

Provided for non-commercial research and education use.
Not for reproduction, distribution or commercial use.



This article appeared in a journal published by Elsevier. The attached copy is furnished to the author for internal non-commercial research and education use, including for instruction at the authors institution and sharing with colleagues.

Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited.

In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier's archiving and manuscript policies are encouraged to visit:

<http://www.elsevier.com/authorsrights>



Contents lists available at SciVerse ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Transfer of metals and metalloids from soil to shoots in wild rosemary (*Rosmarinus officinalis* L.) growing on a former lead smelter site: Human exposure risk

Marie-Cécile Affholder^{a,*}, Pascale Prudent^a, Véronique Masotti^b, Bruno Coulomb^a, Jacques Rabier^b, Bénédicte Nguyen-The^{c,d}, Isabelle Laffont-Schwob^b

^a Aix-Marseille Université, CNRS, LCE, FRE 3416, 3 pl. Victor Hugo, case 29, 13331 Marseille, France

^b Aix-Marseille Université, CNRS, IMBE, UMR 7263/IRD 237, 3 pl. Victor Hugo, case 4, 13331 Marseille, France

^c Montpellier Supagro, UMR MISTEA, 2 pl. Pierre Viala, bat. 29, 34060 Montpellier CEDEX 2, France

^d INRA, UMR 0729 MISTEA, 2 pl. Pierre Viala, 34060 Montpellier CEDEX 2, France

HIGHLIGHTS

- Metal & metalloid contamination of soils & plants from a former smelting factory.
- Low metal and metalloid transfer from soil to rosemary edible aerial parts.
- Low contaminants transfer into herbal tea, below drinking water regulation level.
- Restricted health risk associated with ingestion of contaminated rosemary.
- More antioxidant compounds in essential oils obtained from contaminated rosemary.

ARTICLE INFO

Article history:

Received 13 November 2012

Received in revised form 24 February 2013

Accepted 25 February 2013

Available online xxx

Keywords:

Aromatic plant

Essential oils

Health risk

Herbal tea

Trace elements mixed contamination

ABSTRACT

This study aimed at estimating exposition risks to wild rosemary used as herbs in the contaminated area of the former smelting factory of L'Escalette (South of Marseille, France). Metals and metalloids *i.e.* Pb, As, Sb, Zn, and Cu concentrations were analyzed in soils and in rosemary aerial parts (stems and leaves) on two sites: one heavily contaminated and the other far away from the pollution source, considered as reference. The metal and metalloid transfer into water during the brewing process of herbal tea was also determined. A mixed contamination by the above-cited contaminants was demonstrated in soils of the factory site, with average concentrations of 9253, 1127, 309, 2698 and 32 mg/kg for Pb, As, Sb, Zn and Cu, respectively. However, metals and metalloids' transfer in rosemary aerial parts was limited, as bioaccumulation factors were under 1. Thus, Pb, As and Cu concentrations in leaves were below international regulation limits concerning ingestion of medicinal herbs (no regulation values available for Sb and Zn). This study highlighted that, if contaminated rosemary leaves were ingested, health risks may be limited since acceptable daily intake (ADI) for Pb, As, Sb and Cu (no ADI value available for Zn) will only be reached if very high quantities are consumed. Furthermore, we aimed to establish if this mixed contamination could alter rosemary's essential oil quality, and thereby the compositions of essential oils obtained from individuals on the heavily contaminated soil were compared to those obtained from the reference population. An increased biosynthesis of antioxidant compounds was favored in essential oils from rosemary individuals growing in contaminated site. Although the health risk of a long-term exposition of low level of the mixed contamination by rosemary ingestion is not easy to elucidate, the use of rosemary essential oils from contaminated site appears as safe.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

Rosmarinus officinalis L., known as rosemary, is a perennial plant species, growing wild in the Mediterranean Basin (Zaouli et al., 2003). Due to its antibacterial and antioxidant activities, rosemary essential oils are widely used in pharmaceutical, cosmetic and food industry (Al-Sereitia et al., 1999; Cheung, 2007). Moreover, Mediterranean people commonly use it for cooking as herb, as well as herbal tea and some of them use its essential oil for therapeutic treatments amongst antispasmodic, diuretic

* Corresponding author at: Université Aix-Marseille, CNRS, Laboratoire de Chimie de l'Environnement (FRE 3416), Equipe: Développement Métrologique et Chimie des Milieux (DMCM), 3 place Victor Hugo, case 29, 13331 Marseille CEDEX 3, France. Tel.: +33 4 13 55 10 36; fax: +33 4 13 55 10 60.

E-mail addresses: marie-cecile.affholder@univ-amu.fr (M.-C. Affholder), pascale.prudent@univ-amu.fr (P. Prudent), veronique.masotti@imbe.fr (V. Masotti), bruno.coulomb@univ-amu.fr (B. Coulomb), jacques.rabier@imbe.fr (J. Rabier), benedicte.fonze@supagro.inra.fr (B. Nguyen-The), isabelle.laffont-schwob@imbe.fr (I. Laffont-Schwob).

and choleric activities (Lopez-Munoz et al., 2006). During the middle age, rosemary was thought to possess magical properties. Indeed, in the 14th century, this plant was recorded to be one of the principal components of the Queen of Hungary water formula, thought to maintain the youthful appearance far beyond the normal span of time (Sullivan, 1994).

In Provence (South-East of France), people harvest aromatic herbs and particularly, rosemary, directly from the countryside, and prepare many meals with them. In fact, rosemary is in part involved in the “French paradox” phenomenon of healthy food (Kaloustian et al., 2000) owing to its richness in phenolic compounds. Rosemary essential oil is also commonly used in food enhancement (Boskou, 2006). However, in this geographical area, past industrial activities of the XIXth century lead to many ruins and polluted wastelands that were surrounded by matorrals and/or on which a spontaneous re-colonization by the typical vegetation of matorrals occurred.

In the peri-urban area of the French Mediterranean city of Marseille, in the enclosure of the National Park of Calanques (south of Marseille), the former smelting factory of L'Escalette converting galena into lead and silver is a typical example of the current incidence of past industrial activity (referenced in the French database of polluted soils BASOL, 2006). Heavy metals and metalloids (MM) from this industrial activity were detected all over the Marseillevyre hills (Laffont-Schwob et al., 2011; Testiati et al., 2013). In 1925, the silver–lead ore smelting activities ceased. It has been evaluated that many thousands of m³ of slag heaps and rubbles are still present in the valley of L'Escalette but it is difficult to assess the extent of pollution dispersed all around the industrial site. However, this area is inhabited and these pollutants may cause health risk to humans (Lasalle, 2007). Several wild native plant species, including rosemary, have colonized the contaminated industrial site and the surrounding area.

Previous studies evidenced MM transfer to the aerial parts of rosemary (Cala et al., 2005; Abdul-Wahab et al., 2008; Sekeroglu et al., 2008). Thus, consumption of edible parts contaminated by these toxic elements may induce a risk for human health. Translocation of heavy metals from soil to aerial parts of rosemary, particularly in the case of mixed pollution as in L'Escalette surroundings, has to be evaluated depending on soil contamination. Previous studies highlighted that no transfer of heavy metals was observed in essential oils obtained by steam distillation of various plant species (Zheljzkov et al., 2006, 2008a, 2008b). Thus, an interesting alternative of using rosemary from the contaminated area without health risk could be the use of its essential oil. Furthermore, rosemary essential oil composition, and also its antioxidant properties, are linked to soil characteristics (Ormeno et al., 2008) and plant development stages (Del Bano et al., 2003), so the possible alteration of the chemical composition of the essential oil needs to be investigated.

This study aimed at determining exposition risks for the local population picking rosemary for cooking and herbal tea consumption in the contaminated area of L'Escalette. Furthermore, it aimed at highlighting if MM contamination could alter rosemary essential oil quality by repressing or enhancing some compound biosynthesis. Therefore, we first analyzed MM transfer from a representative heavily contaminated soil to the aerial part of rosemary *in natura* as well as their transfer into water during brewing process and compared it to a population growing far away from the pollution source, considered as reference. Secondly, compositions of essential oils obtained from plant grown on the heavily contaminated soil were compared to those obtained from the reference population.

2. Material and Methods

2.1. Study Area

Two sites were selected for this study so that they have very similar soil composition, vegetation and climatic conditions. These sites, located in the peri-urban area of Marseille, in South-East France, were

characterized by a matorral vegetation dominated by *Rosmarinus officinalis*, *Cistus albidus*, *Quercus coccifera* and *Pistacia lentiscus* under Mediterranean climatic conditions. The site A (43°13.583' N; 5°21.322' E), so called “polluted site”, was located in the vicinity of the former factory of L'Escalette (close to the chimney exit) and the site B (43°14.075' N; 5°23.197' E), in the suburban area of Marseille, in the never industrialized part of the Calanques hills, so called “reference site” (Fig. 1). Activities at L'Escalette lasted from 1851 to 1925 and during this period around 30,000 t/year of silver–galena were treated by pyrometallurgical processes. The factory, situated at the lower part of a hill, is characterized by a horizontal chimney (creeping chimney) built up on the hillside. Slags have been deposited on the factory site or nearby. The climate is characteristic of the Mediterranean areas with average minimal and maximal temperatures of 7.1 and 24.1 °C, respectively, and an annual average rainfall of 554 mm during 56 days (reference period: 1971–2000). Dominant winds are North and North-East and can reach 120 km/h. The geological substrate is limestone and bare rocks dominate. The soils of selected sites were stony; their thicknesses varied from place to place, but were generally less than 50 cm. Soils were alkaline with pH (ISO 10390, 2005) between 7.7 and 8.2, belonging to the typical pH range of soils from calcareous areas (Fernández-Ugalde et al., 2011). Soil fertility was low with total organic carbon contents varying from 3 to 7%, total Kjeldahl nitrogen (ISO 11261, 1995) contents from 3 to 7 mg N/g, assimilable phosphorous contents (ISO 11263, 1995) from 0.01 to 0.04 mg P/g, a cation exchange capacity (CEC, ISO 23470, 2007) varying from 15 to 30 cmol⁺/kg, and clay contents varying from 10 to 27%. The soils collected in site A presented CEC and clay contents in the top part of the range of values measured in the Calanques hills area, and the soils collected in site B were in the middle range.

2.2. Plant and Soil Sampling

Sample collection was done on five rosemary individuals of a same population on each site. In order to obtain representative samples of each site, an area of 100 m² (10 × 10 m) was delimited on each site. The plant cover in both sites was over 60% on the selected areas, *R. officinalis* populations corresponded to 15 to 20 old and large individuals. The 5 rosemary individuals were selected according to a cross pattern inside the delimited area, spaced by around 2 m, with similar sizes, *i.e.* heights and collar diameters, and same phenological stage. Therefore, on each site, a total of 5 plant/soil couples were taken. Stems and leaves were collected for metals and metalloids (MM) and for essential oil analyses. Soil samples were collected from the top 15 cm (after removal of the litter) in the rhizospheric area of the plants. Fresh plant and soil samples were stored in clean plastic bags for transport.

2.3. Soil MM Analysis

Soil samples were sieved on site to 2 mm mesh, air-dried at room temperature and then ground (RETSCH zm 1000 with tungsten blades) to pass through a 0.2 mm titanium sieve before analyses. Soils were mineralized in a microwave mineralizer (Milestone Start D) using *aqua regia* (1/3 HNO₃ + 2/3 HCl). The mineralization products were filtered with a 0.45 μm mesh and the metal levels were determined by ICP-AES (Jobin Yvon Horiba, Spectra 2000) for Zn, Cu, Fe, Mn and Pb (Lotmani et al., 2011), and by graphite furnace AAS (Thermo Scientific ICE 3000) for As and Sb. Quality assurance–quality controls and accuracy were checked using standard soil reference materials (CRM 049–050, from RTC-USA) with accuracies within 100 ± 10%.

2.4. Plant MM Analysis

Rosemary stems and leaves were washed separately with Milli-Q water to remove possible attached particles. All samples were dried at 40 °C during one week and then ground at 0.2 mm (RETSCH zm 1000

blender). About 0.5 g of dry matter was digested with the microwave digestion system Milestone Start D with a HNO₃–HCl mixture (volume proportion ratio 2/1). Extracts were analyzed for Cu, Pb, Zn contents by ICP-AES, and for As and Sb by graphite furnace AAS (Rabier et al., 2007). Standard plant reference materials (DC 73349) from China National Analysis Center for Iron and Steel (NCS) were analyzed as a part of the quality assurance–quality control protocol (accuracies within 100 ± 10%). Bioaccumulation factors (BAFs), i.e. ratios of shoot concentration vs. soil concentration, were calculated.

2.5. Hydrodistillation and Essential Oil Analysis

2.5.1. Isolation of the Essential Oils

Oil samples were isolated from freshly air-dried (room temperature) leaves, by hydrodistillation for 2 h using a Clevenger-type apparatus as previously described (Schwob et al., 2004). For each plant, a separate essential oil (EO) sample was obtained, i.e. five EO per population. EO yields were then estimated on the basis of the dry weight of plant material.

2.5.2. Chemical Analysis of the Essential Oils

2.5.2.1. Gas Chromatography (GC). GC analyses of the EO were performed on a GC instrument (Varian, model 3900GC) with a flame ionization detector (FID), equipped with a CP SIL 8CB fused silica capillary column (30 m × 0.25 mm, 0.25 μm film thickness). Oven temperature was programmed from 50°–200 °C at 3 °C/min, after an isothermal step at 50 °C for 2 min. The carrier gas was hydrogen, with a flow rate of 0.5 mL/min. Split–splitless injector and detector were heated to 220 °C and 230 °C, respectively. Essential oil solutions in n-pentane (5%) were injected in split mode (1:30).

2.5.2.2. Gas Chromatography Coupled with Mass Spectrometry. GC/MS analyses were also carried out on a GC/MS Hewlett-Packard (Model 5972) chromatograph equipped with a DB5 fused silica capillary column (25 m × 0.2 mm, film thickness 0.25 μm) coupled with a quadrupole mass spectrometer. Temperature program was 3 °C/min from 60 to 220 °C. Split–splitless injector and detector were heated to 220 °C and 270 °C, respectively. Helium was used as carrier gas at a flow rate of 1 mL/min. The instrument was operated under electronic impact ionization with 70 eV as ionizing energy. Total ion current (TIC) data were collected with a scan time of 0.1 s over a mass range of 38 to 400 Da.

2.5.3. Identification of Components

Individual components were identified by comparison of both mass spectra and GC retention data with those of authentic compounds previously analyzed and stored in the data system (computer matching with the NBS 98K and WILEY 275 libraries). Other identifications were made by comparison of mass spectra with those in the data system libraries and cited in the literature (Adams, 2001). The retention indices were calculated for all volatile constituents using a n-alkanes homologous series (Jennings and Shibamoto, 1980).

2.6. Herbal Tea

Herbal teas were prepared according to common consumer practices. Therefore, floral heads were collected on each rosemary individual, rinsed with tap water and then oven-dried at 35 °C during 3 days. Stems were removed to only keep the leaves. Volume prepared was 250 mL herbal tea for each rosemary sample, with a concentration of 50 g dry leaves/L. Infusions were made with a low mineralized natural spring water (pH: 5.85, dry residue: 25 mg/L). Boiling water was added to the leaves and brewed during 15 min. Leaves were then removed, and herbal tea filtered at 0.45 μm. Solutions obtained were

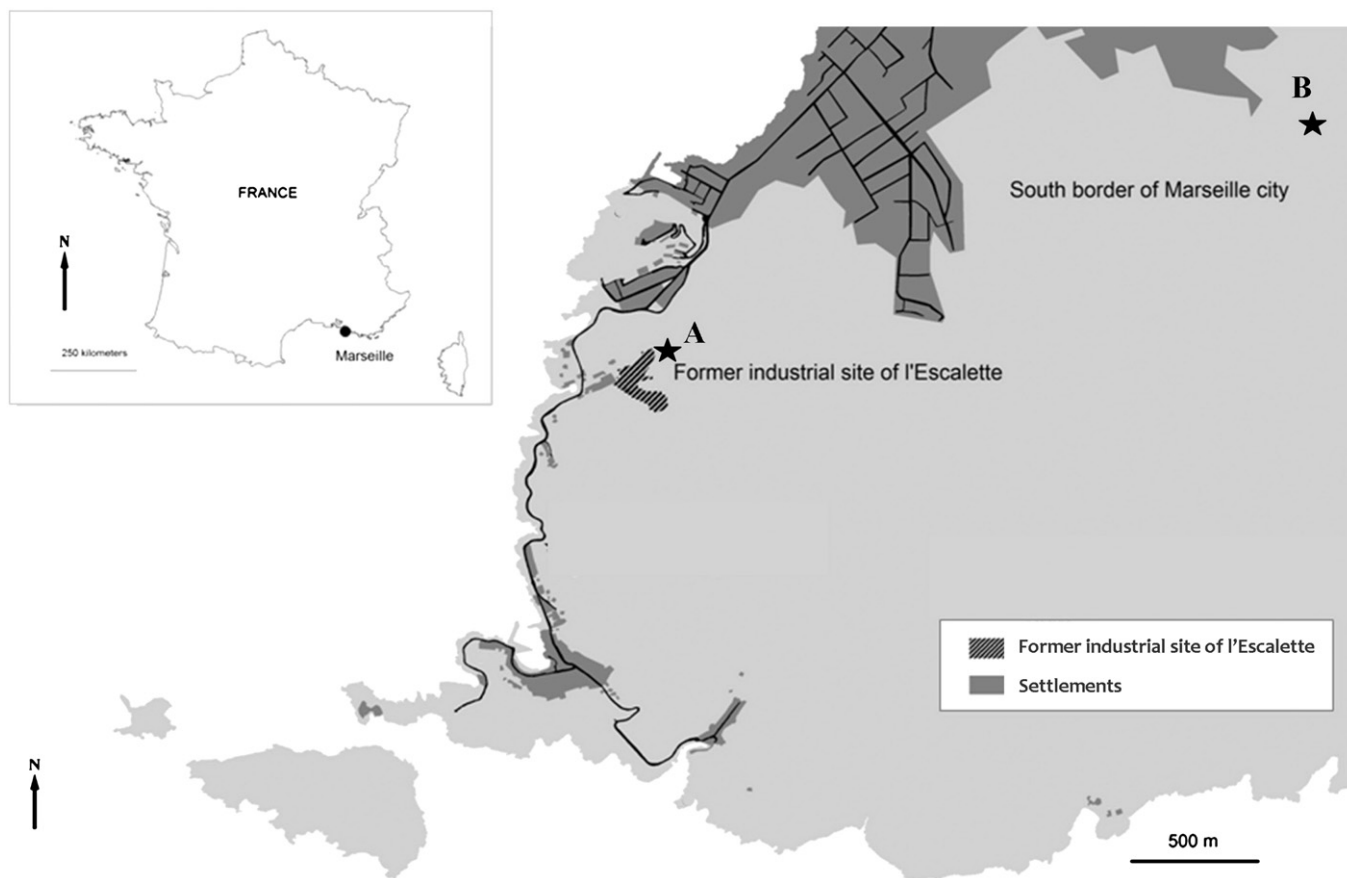


Fig. 1. Localization map of the studied sites (A: contaminated site and B: reference site).

then acidified at pH 4 with HNO₃ (Supra-pur) and stored at 4 °C until analysis. MM were measured by ICP-AES for Cu and Zn, and by GF-AAS for As, Pb and Sb.

2.7. Statistical Analysis

Statistical analyses were performed with R. A rank based Wilks' lambda statistic was used as proposed by Nath and Pavur (1985) to test the equality of the group means of A and B soil properties, soil MM concentrations, shoot bioaccumulation, herbal tea MM concentrations and average chemical composition of essential oils (function Wilks' test, package rr covrank). Heterogeneous covariance matrices were observed between the two groups, but as stated by Finch (2005), "early research found that when the groups did not differ in size, the effect of heterogeneous covariances is very small". A two factor Manova, with a label permutation test, was performed (Manly, 1997) to test interaction and simple effects on MM concentrations from populations (A and B) and from organs of rosemary (leaves and stems). Post hoc mean comparisons were carried out with nonparametric test, Wilcoxon, using Bonferroni correction.

To study the links of MM concentration table with the composition of essential oil table, a co-inertia analysis (Dray et al., 2003) was performed with package Ade4. This analysis consists of two separates PCA (one for each table). The first principal component axis of each PCA is then rotated so that the covariance between the two sets of projected scores is maximal, and so on. In addition, the RV coefficient (Robert and Escoufier, 1976) was used to measure the closeness between the MM concentrations and the composition of essential oil data matrix. This coefficient is a multivariate generalization of the squared Pearson correlation coefficient. The permutation test of association between configurations by means of the RV coefficient suggested by Heo and Gabriel (1997), available from package Ade4, was conducted with $n = 10,000$ permutations.

3. Results and Discussion

3.1. MM in Soils and Rosemary

MM concentrations were significantly higher in soil samples collected in site A compared to B (Table 1) despite the high heterogeneity observed between the soil samples from a same site, especially at the contaminated site, located near the creeping chimney exit. These observations concerned particularly some elements such as As, Pb, Sb and Zn. Indeed, relative standard deviations (RSD %) exceeded 50% for the contaminated soil samples analyzed. This is typical of anthropogenic contamination and particularly contamination with atmospheric deposit from industrial activities (Yaylali-Abanuz, 2011; Testiati et al., 2013). Contamination relative to the former smelting factory of L'Escalette concerned especially xenobiotics elements such as As, Pb and Sb for which the ratios of A to B concentrations were higher than 200. Soil concentration ratio between site A and B was about 50 for Zn and stays under 5 for Cu, Fe and Mn. The presence of these MM in site A could be linked to the composition of silver–galena ores treated in the former factory, which contain high levels of Pb, Fe, Zn, Sb and As (Palero-Fernandez and Martin-Izard, 2005). Thus oligo-element (Zn and Cu) and soil major element (Fe and Mn) variation was less important. No information are available on the MM natural geological background of the studied areas, but, a mapping of element concentrations in an extended area around the factory site was conducted. This zone was divided into 250 squares all over the Marseilleveyre hills, with a mesh size of 250 × 250 m each. On the center of each square, a top-soil sample (0–15 cm depth) was collected from a mesh size of 1 m². For all these 250 samples, MM contents were determined with a portable X-ray fluorescence spectrometer (Niton XLT 792, Fondis electronic). The numerous data obtained were compiled into a database, and their analyses lead to an estimation of the local contamination background level by comparing the 1st percentiles 0.05 ($n = 250$) that are of <50, <10, 40, <50 and 53 mg/kg for As, Cu, Pb, Sb and Zn, respectively. Results clearly evidenced high contamination

Table 1

Average MM concentrations (mg/kg) in soil samples from each site (A: contaminated site and B: reference site).

Element type	Element concentrations (mg/kg) in each site	
	Site A	Site B
As	1127 ± 836 a	5 ± 2 b
Cu	32 ± 5 a	7 ± 3 b
Fe	20,204 ± 2582 a	10,486 ± 1954 b
Mn	551 ± 39 a	340 ± 54 b
Pb	9253 ± 5540 a	41 ± 17 b
Sb	309 ± 230 a	1.5 ± 0.5 b
Zn	2698 ± 1677 a	55 ± 22 b

Means (± standard deviation) followed by different letters in the same line are significantly different (Wilcoxon test, $\alpha = 0.05$, $m = 7$, Bonferroni: significant p -value < 0.007) after a one-way MANOVA: rank Wilks' lambda = 0.0163, Chi2-value = 18.519, DF = 7, p -value = 0.0098.

of the site A, and this, for every element analyzed. Concentration values for these same MM were below the estimated background level in the site B, confirming its legitimacy as reference soil. To our knowledge, only few guidelines concerning soil contamination are available, but these concentrations could also be compared to some National thresholds for heavy metals and to the predictable non-effect concentration (PNEC) values for soils (Table 2). No values are available for Sb. The reference soil MM concentrations were below National thresholds, but some of them were above PNEC values. For the site A, only copper values are below the National threshold. Moreover, the high contamination was again highlighted with concentration ratios between the site A and PNEC values higher than 500, 700, 100 and 10 for As, Pb, Zn and Cu, respectively.

Fig. 2 shows MM results in rosemary stems and leaves for each area. Only oligo-elements and xenobiotic absorptions by rosemary are discussed in this paper. The MANOVA global test indicated no significant interaction or organ (stem, leaf) effects. Only the site effect was significant (p -value < 10⁻³). A high heterogeneity was observed between stems and leaves from the site A notably for As, Pb and Sb for which some high and low outlier values were highlighted. Rosemary leaves being used for culinary and medicinal purposes, it was important to check if MM concentrations in these plant parts from field were below the food regulation level. Only few regulations exist concerning rosemary use as herbs and we refer to the National limits (WHO, 2007) and the European Pharmacopoeia (Herbal drugs monograph, 2008) concerning toxic metals in herbal products for As, Pb and Cu only. Proposed limits are 5 mg/kg for As (Canada, Malaysia and Singapore) (WHO, 2007) and Pb (Herbal drugs monograph, 2008) and 150 mg/kg for Cu (Singapore).

Concerning As contamination, only stems showed a significant difference depending on the site. For Cu, stems' and leaves' results showed no significant differences between concentrations in rosemary individuals

Table 2

Russian (from a neutral soil i.e. pH > 5.5), Netherlands (as reference value), Canadian (from an industrial zone) and Turkish thresholds for MM in soils and PNEC values expressed in mg/kg (dry soil).

Elements	Concentrations of elements in soil (mg/kg dry soil) from different sources				
	Russian ¹	Netherland ¹	Canadian ¹	Turkey ²	PNEC ³
As	10	20	50	–	1.8
Cu	–	–	–	140	12
Pb	–	–	–	300	21
Sb	–	–	–	–	–
Zn	220	–	800	300	2.7

¹ Komnitsas and Modis (2006, 2009).

² Yenilmez et al. (2011).

³ INERIS (2010, 2005a, 2005b, 2003).

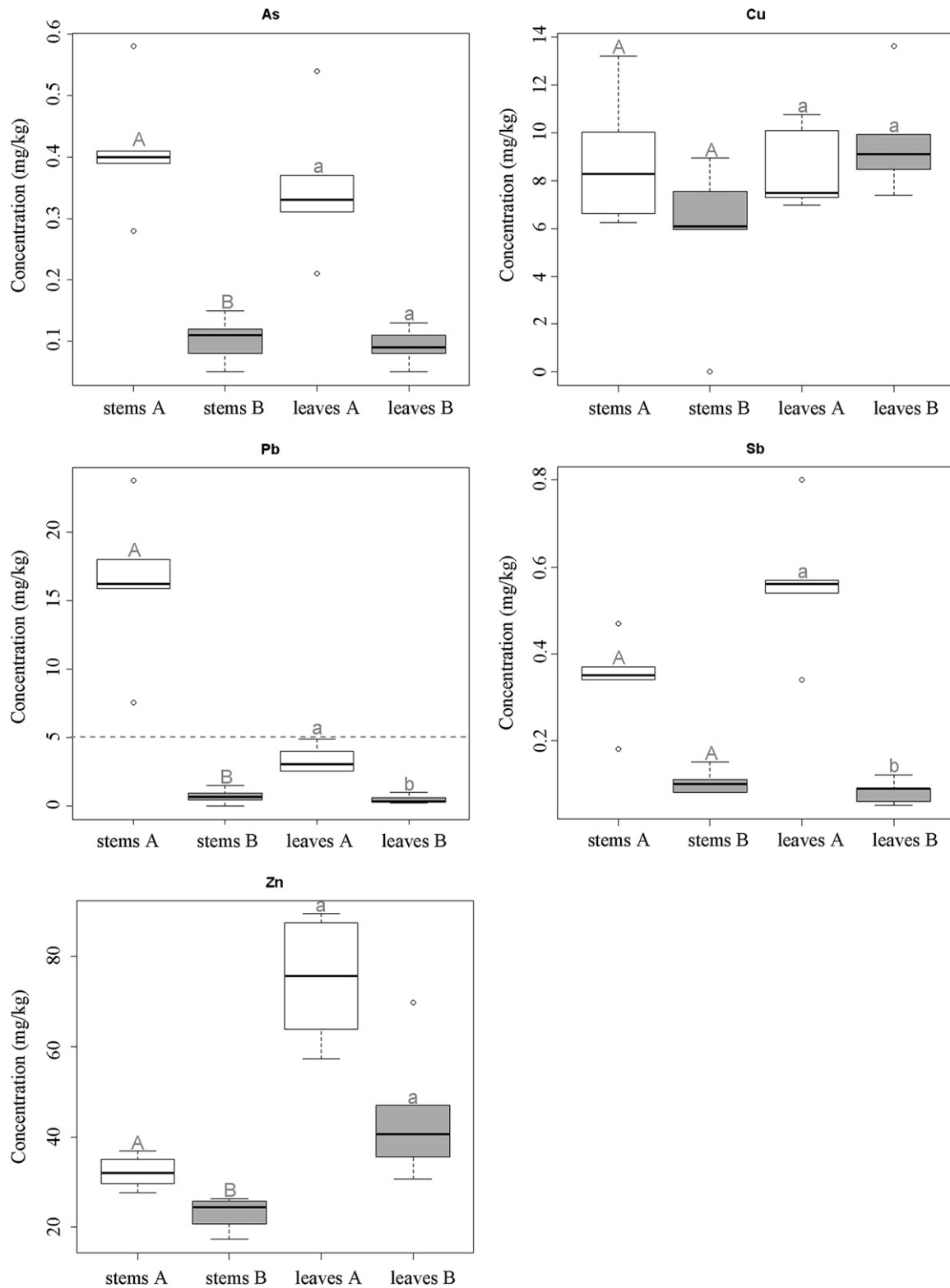


Fig. 2. Concentrations (mg/kg) of As, Cu, Pb, Sb, Zn in different organs of rosemary (leaves and stems) for each site (A in blank, contaminated site; and B in gray, reference site). Two-way MANOVA: simulated p-values for interaction = 0.146, organ = 0.056, population $< 10^{-3}$. Statistical significant difference (Wilcoxon test; $\alpha = 0.05$, $m = 10$, Bonferroni: significant p-value < 0.005) is represented by different letters above each boxplot (capitals for stems and lower-case letter for leaves). Dotted line represents regulation limit for Pb, not represented for As (5 mg/kg) and Cu (150 mg/kg) since values are not in the scale interval.

from A and B, this could be explained by the low level of Cu soil content and because Cu is an essential element to plants. For both latter elements, all the concentration results in leaves of rosemary individuals collected in the contaminated site were largely below the permissible limits.

Concerning Pb, for both plant parts, concentrations were significantly higher in rosemary from site A compared to site B. Pb concentrations in stems from rosemary individuals collected in site A reached a mean of around 16 mg/kg. However, this element was less concentrated in the corresponding leaves, i.e. the mean value was about 3.5 mg/kg, with a

maximum reaching 4.9 mg/kg. This latter value was close to the European pharmacopoeia (Herbal drugs monograph, 2008) limits concerning Pb i.e. 5 mg/kg in herbal products. Moreover, concentrations measured in all the stems from rosemary individuals collected in the site A were over this permissible limit. Thus, Pb exposure risk for the local population collecting rosemary growing around the smelter factory seemed low,

with the advice to use leaves without stems. Sb concentrations in leaves collected in individuals from the site A were higher than those from the non-contaminated reference site. No significant difference was highlighted concerning stems' concentrations. For Zn, results showed concentrations significantly higher in stems from rosemary grown wild on the site A compared to those on the site B, while concentrations in leaves

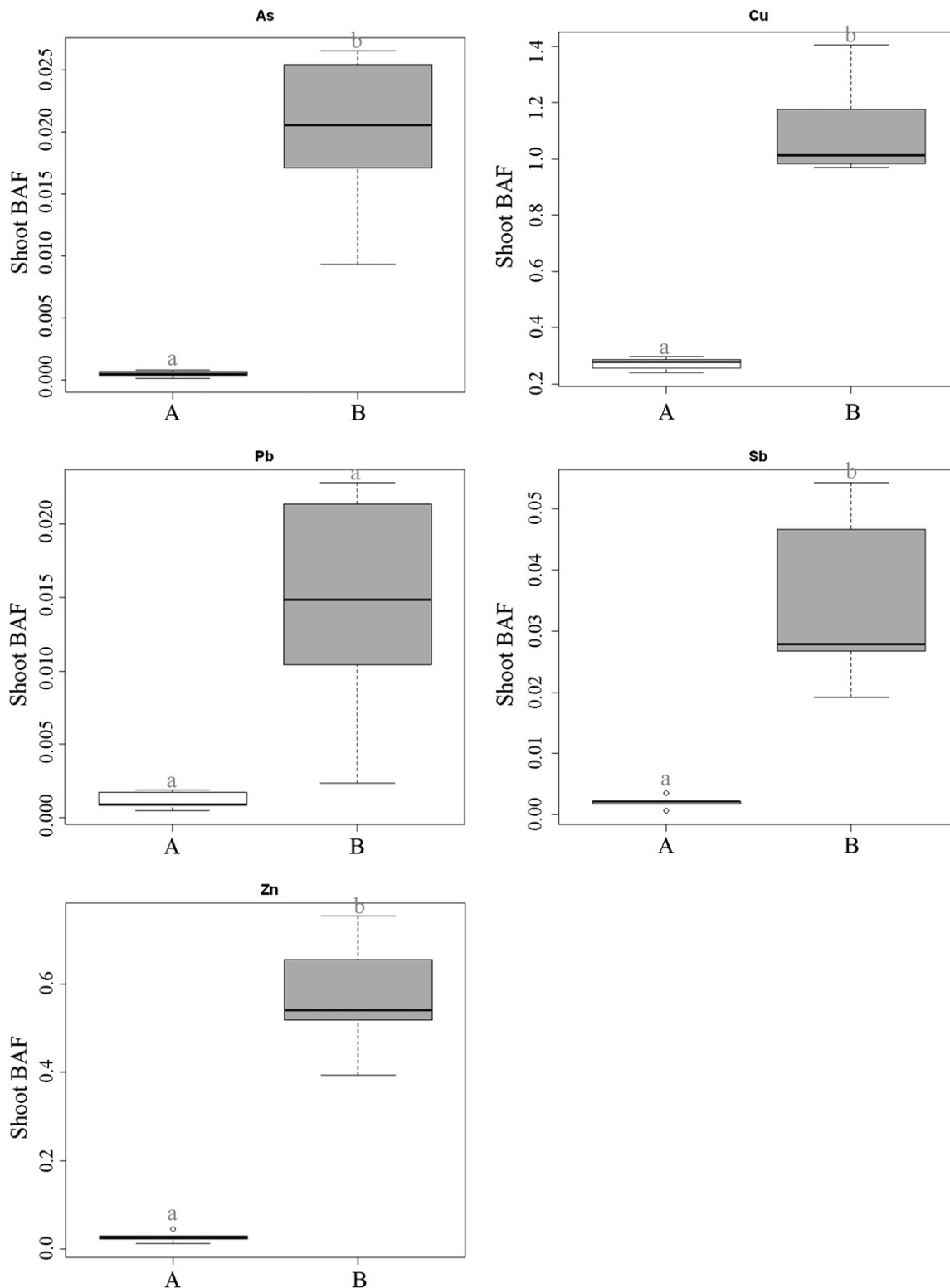


Fig. 3. Rosemary shoot bioaccumulation factors for As, Cu, Pb, Sb and Zn on each location (A in blank, contaminated site; and B in grey, reference site). One-way MANOVA: rank Wilks' lambda = 0.044, Chi2-value = 17.179, DF = 5, p-value = 0.004172. Significant difference (Wilcoxon test; $\alpha = 0.05$, $m = 5$, Bonferroni: significant p-value < 0.01) is represented by different letters above each boxplot.

did not differ significantly. Zn was preferentially stored in leaves in agreement with the involvement of this element in plant respiration as enzyme co-factor (Todeschini et al., 2011). In the observed rosemary individuals, Zn levels in leaves were not significantly different for both sites. Previous researches showed that Zn translocation could be modulated by mycorrhizal associations (El-Kherbawy et al., 1989) and our own study highlighted that roots from rosemary individuals collected on the site A, with significantly higher Zn levels, were indeed highly endomycorrhized compared to those from the site B (results not shown).

Bioaccumulation factors for As, Cu, Pb, Sb and Zn were calculated for each site (Fig. 3). Results showed significantly higher BAFs for rosemary individuals growing on the site B compared to those on the site A, and this, for all the oligo-elements and xenobiotics studied. Shoot BAF levels were very low, particularly for plants from the site A, i.e. average BAFs for A were 0.0005, 0.014, 0.0012, 0.002 and 0.027 for As, Cu, Pb, Sb and Zn, respectively. Except for Cu in the non-contaminated site, rosemary shoot BAFs were lower than 1, highlighting that this plant species was not an accumulator *sensu* Baker, 1981. Moreover, inorganic uptake by plant depends on a discrete number of membrane proteins, saturable following Michaelis-Menten kinetics (Pilon-Smits, 2005). Thereby, lower BAFs in rosemary from the site A could be explained by uptake saturation related to the important mixed MM concentrations on this site. This uptake saturation hypothesis is reinforced by the results of Domiguez et al. (2008). Thus, MM concentrations in rosemary aerial parts in this latter study were of the same order than those we obtained, since the authors found 0.79, 13.2, 2.01, 0.046 and 51.2 mg/kg for As, Cu, Pb, Sb and Zn, respectively in rosemary leaves collected in a spill-affected site. However, soil contamination of this authors' site was lower than in L'Escalette. Another explanation could be linked to the phytodisponibility of MM in soil from site A. Indeed, total MM concentrations are generally not representative of concentrations really available to be taken up by plants (Chojnacka et al., 2005). Thus, other extraction solvents must be used i.e. EDTA (Quevauviller et al., 1997), calcium nitrate (Quevauviller, 1998) or ammonium citrate (Chojnacka et al., 2005), aiming to highlight labile and bioavailable MM concentrations.

3.2. Essential Oil Chemical Composition

For all the essential oils, a yield of ca. 1% was obtained in both sites. This yield corresponds to the average yield encountered in the literature i.e. yields from 0.10 to 2.13 using the same process of isolation (Angioni et al., 2004; Boutekedjiret et al., 2003). Chemical compositions were the same for both sites with 46 identical identified components (>98% of total composition) (Table 3). Major components were camphor, α -pinene and 1,8-cineole for both sites enabling to assert both rosemary populations belong to the same chemotype. Chemical composition of essential oils from both populations (contaminated and non-contaminated areas) being the same, it seemed that no variation of regulation of a specific biosynthesis pathway in the terpenoid synthesis was observed depending on pollution.

However, despite the low variation of composition between essential oils obtained from both sites, a co-inertia analysis between essential oil composition and MM was done (Fig. 4). The first axis represented 92.2% of variability, while the second one represented 6.3% of variability. The first two principal component information from respectively oil compositions and MM were well represented in this plane with ratios of 99% and 76% for MM and oil compositions, respectively. It highlighted a link between soil MM contamination and essential oil composition (correlation between metals' and oils' principal components was around 0.7 for co-inertia axis 1 and also for axis 2). Fig. 4 shows that As, Sb, Zn and Pb varied in the same way and were positively associated with linalyl cations and sesquiterpenes, whereas they were negatively associated with lipids and bornyl cations. Cu, Fe and Mn formed another group and this latter was positively associated with phenolic compounds and negatively with sabinyl cations. Information from some oil groups (pinyl cation, 4 terpinyl cation and α -terpinyl cation) was not clearly

related with metals. We projected, as supplementary information, the terpenes detailed components (Fig. 4 in grey). In non-contaminated soils, verbenone, pino-carvone, myrtenol (monoterpenes, pinyl cation) and sabinene (monoterpene, sabinyl cation) seemed more represented. These latter were best known as odorous components of the volatile essence of flowers and of the essential oils of herbs. They were considerably used as flavors and perfumes (Croteau et al., 2000). Some compound likes myrcene (monoterpene, linalyl cation), β -farnesene and β -caryophyllene (two sesquiterpenes) seemed linked to As, Pb, Sb and Zn. As a primary stress response of plants exposed to MM, reactive oxygen species (ROS) may be generated (Yadav, 2010), although antioxidants' capacities depend on compound reducing abilities (Apak et al., 2007). Presence of methylation in sesquiterpenes, found in higher quantity in rosemary essential oils from the contaminated site, seemed to confer them a high reducing potential (Ben Sghaier et al., 2011), and therefore better antioxidant properties. Both kinds of biomolecules were classified as terpenoids, but sesquiterpenoids were mainly produced in the cytosol *via* the acetate mevalonate pathway, and monoterpenoids arising in the plastids *via* the still not totally understood methylerythritol phosphate independent pathway (Davis and Croteau, 2000). Since two different pathways are involved in monoterpenoids and sesquiterpenoids synthesis, observed chemical composition alterations may not be only linked to MM oxidation but also to an enzymatic regulation.

Table 3

Average chemical composition of essential oils from both populations (A: contaminated site and B: reference site) expressed as percentage. Major compounds in bold.

Compounds	Percentage of each compound in each population	
	A*	B*
α -Thujene	0.3 ± 0.2	0.3 ± 0.1
α-Pinene	20 ± 6	19 ± 7
Camphene	10 ± 2	8 ± 2
Thuja 2,4 diene	0.3 ± 0.1	0.14 ± 0.12
Octen-3-one	1.4 ± 0.6	1.6 ± 0.2
β -Pinene	0.07 ± 0.07	0.25 ± 1.19
1-Octen-3-ol	0.02 ± 0.03	0.08 ± 0.10
Myrcene	11 ± 7	6.9 ± 7.2
Sabinene	0.06 ± 0.04	0.17 ± 0.07
α -Phellandrene	2 ± 1	0.76 ± 0.63
<i>o</i> -Cymene	0.44 ± 0.95	0.01 ± 0.00
1,8-Cineole	11 ± 5	14 ± 6
Limonene	6 ± 2	3.7 ± 6.7
δ -3-Carene	0.01 ± 0.00	2 ± 3
E- β -ocymene	0.28 ± 0.22	0.02 ± 0.02
γ -Terpinene	0.4 ± 0.3	0.5 ± 0.2
<i>p</i> -Cymene	2.1 ± 0.6	1.6 ± 0.9
Linalool	0.8 ± 0.7	0.5 ± 0.2
<i>p</i> -Cymenone	0.5 ± 0.2	0.5 ± 0.2
Myrcenol	0.38 ± 0.35	0.7 ± 0.2
α -Campholenal	0.25 ± 0.22	0.3 ± 0.2
Camphor	23 ± 9	26 ± 7
Pinocarvone	0.05 ± 0.05	0.23 ± 0.07
α-Terpineol	4 ± 2	5 ± 2
Terpinen-4-ol	0.45 ± 0.42	0.8 ± 0.1
Pinocamphone	0.4 ± 0.2	0.4 ± 0.2
Verbenone	1.1 ± 0.4	2.2 ± 0.5
Myrtenol	0.9 ± 0.2	1.5 ± 0.3
Borneol	0.09 ± 0.08	0.17 ± 0.10
Z-ocymenone	0.5 ± 0.7	0.05 ± 0.05
Cis-myrtanol	0.03 ± 0.02	0.02 ± 0.01
E-ocymenone	0.07 ± 0.09	0.05 ± 0.06
Trans-myrtanol	0.01 ± 0.00	0.02 ± 0.02
Linalyl acetate	0.44 ± 0.35	0.5 ± 0.3
Bornyl acetate	0.02 ± 0.02	0.23 ± 0.47
Z-3 hexenyl tiglate	0.06 ± 0.08	0.04 ± 0.05
Methyl eugenol	0.25 ± 0.24	0.08 ± 0.13
β -Caryophyllene	0.15 ± 0.20	0.04 ± 0.04
β -Farnesene	1.1 ± 0.7	0.65 ± 0.63
Identified percentage	99.7 ± 0.3	99.7 ± 0.2

* Means of 5 essential oils per population.

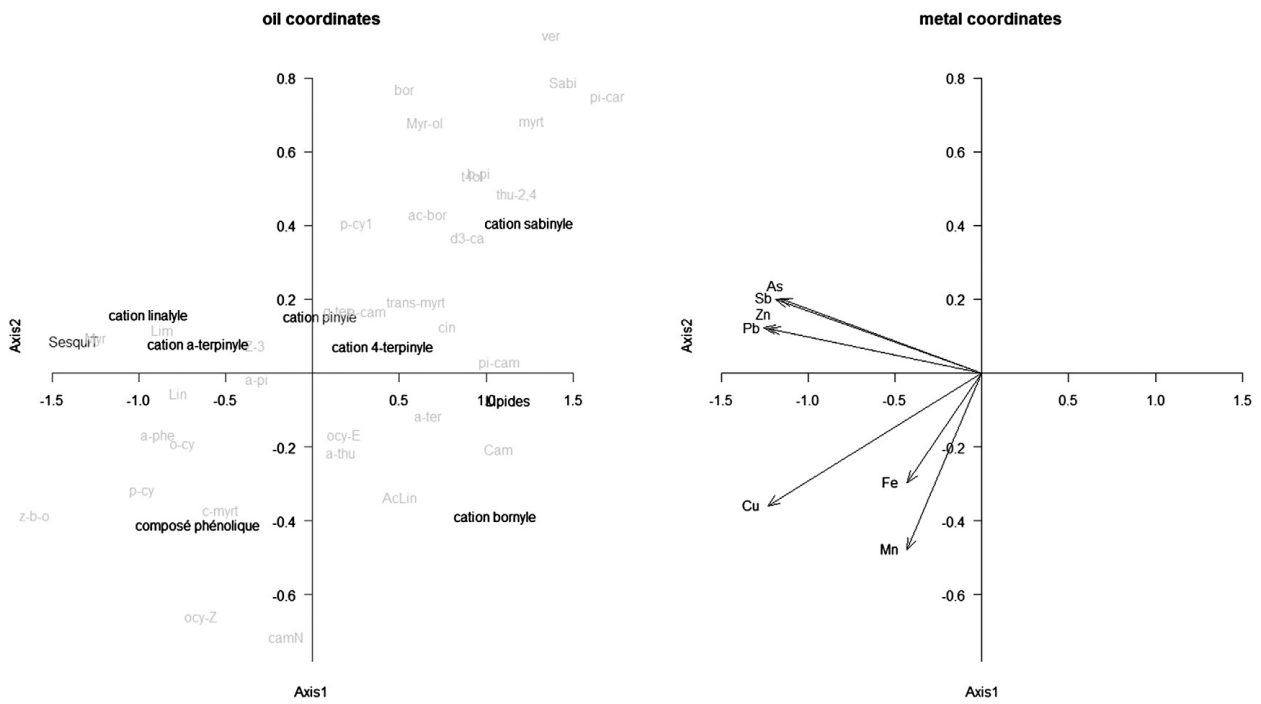


Fig. 4. Coinertia analysis between essential oil chemical composition and MM soil contamination.

3.3. MM in Herbal Teas

MM were analyzed in herbal teas (Table 4). Results obtained with rosemary samples from contaminated and non-contaminated sites were compared with the FAO/WHO guideline concerning drinking water (FAO/WHO, 2008). Our results showed that MM transfer during brewing process was limited. However, there were significantly more As and Sb in herbal teas obtained with rosemary from the site A than from the site B. Contrariwise, Cu concentration in herbal teas was significantly higher when obtained with rosemary individuals from the site B than those from the site A (97 µg/L and 27 µg/L, respectively) while concentrations in leaves were almost the same (9.7 and 8.5 mg/kg, respectively). Calculation of the metal transfer ratio (%) into infusion highlighted that, extraction efficiency was elemental dependent and in most cases, dependent on leaf concentration. Thereby, except for As (transfer ratio about 12), percentages of elements released were significantly different between both sites. Indeed, transfer percentages from rosemary were 6.6, 2.0, 3.4, and 0.64 in herbal teas from the site A, and were about 19.4, 5.5, 10.8 and 2.5 in herbal teas from the site B for Cu, Pb, Sb and Zn, respectively. For these latter elements, except Cu, transfer ratios into herbal teas were proportionally higher when leaf contamination was lower.

Thus, MM concentrations in herbal teas obtained with rosemary leaves from contaminated and non-contaminated areas were below the permissible limits proposed by the *Codex Alimentarius* concerning drinking water (FAO/WHO, 2008) and this, for the 4 elements considered. However, MM speciation in herbal tea has to be taken into account, since toxicity and bioavailability are highly dependent on speciation for some elements such as As (Creelius, 1977), Pb (Oomen et al., 2003) and Sb (Sundar and Chakravarty, 2010).

A previous study showed that in herbal infusions, As was mainly (72–98%) associated with biomacromolecules, in the same way, the main part of Pb forms strong complexes (Aspadjan et al., 2008). It was assumed that the element-binding organic ligand in herbal teas were largely polyphenolic compounds (Toda, 2011). Since polyphenols were reported as poorly absorbable chemical species (Manach et al., 2004), it could be assumed that As and Pb from herbal teas were almost not bioavailable and therefore less toxic.

3.4. Human Risk

Table 5 presents the minimal quantities of leaves (in g) and herbal tea (in L) from both sites (A and B) to consume in order to reach the acceptable daily intake (ADI, Baars et al., 2001; WHO, 2006) for As, Cu, Pb and Sb (no values for Zn). For As, Cu and Sb in both sites, dry leaves quantity necessary to exceed recommended limits was above 300 g per day, and volumes of herbal tea was above 40 L per day. We could notice the particularity for Pb in the site A with a quantity of dry leaves corresponding to only 51 g per day. Thus, in all cases, masses and volumes were high, and overtake widely the quantity a

Table 4

MM concentrations in rosemary herbal tea in µg/L (A: contaminated site and B: reference site).

Site	Elements µg/L				
	As	Cu	Pb	Sb	Zn
A	2.7 ± 1.1 a	27 ± 7 a	3.7 ± 1.5 a	0.9 ± 0.3 a	23 ± 10 a
B	0.35 ± 0.06 b	97 ± 23 b	1.1 ± 0.3 a	0.42 ± 0.06 b	28 ± 6 a
Reference FAO/WHO regulation*	10	1000	10	5	–

Means (± standard deviation) followed by different letters in the same column are significantly different (Wilcoxon test, $\alpha = 0.05$, $m = 5$, Bonferroni: significant p -value < 0.01) after a one-way MANOVA: rank Wilks' lambda = 0, Chi2-value = 406.559, DF = 5, p -value < $2.2e^{-16}$.

* FAO/WHO *Codex Alimentarius* commission, 2008.

Table 5

Minimal quantities of rosemary leaves (g of dry weight, DW) or herbal tea (L) to consume to reach acceptable daily intake (ADI) for As, Cu, Pb and Sb (no value available for Zn; A: contaminated site and B: reference site).

	Site	Elements			
		As	Cu	Pb	Sb
ADI (mg/day) for a 60 kg adult		0.12 ^a	8.4 ^a	0.21 ^a	0.36 ^b
Leaves (g DW)	A	342	900	51	643
	B	1318	866	437	4500
Herbal tea (L)	A	44	314	57	387
	B	343	87	180	857

^a Baars et al. (2001).

^b WHO (2006).

consumer may ingest. Although for herbal, the European Pharmacopoeia recommends to drink 250 to 500 mL of a 5 g/L rosemary tea, daily. This suggests that MM exposure risk for people picking and eating rosemary in Calanques hills, and notably in the contaminated area, is low, if nonexistent in the case of non-exceptional consumption, considering separately every element. However, health risks could not be completely excluded since Pb is cumulative in body. As reviewed by Pounds et al. (1991), Pb may be stored in bones, substituting calcium on its receptors. This stored Pb could be remobilized in the case of increasing bone turnover, due to osteoporosis for example, with lead intoxication as a consequence (Berlin et al., 1995). Moreover, nothing is known about long time exposition effects to the cocktail of these toxic elements even at low concentrations and this represents a challenge for future research.

4. Conclusion

Under our field conditions, MM accumulation in *R. officinalis* aerial parts was limited, as bioaccumulation factors (BAFs) were under 1 for all MM studied, except for Cu in the non-contaminated site. However, more experimentations have to be carried out to highlight if rosemary is really not able to accumulate MM in its aerial parts or if it is the occurrence of a low phytoavailability of MM in soils. The fact remains that in this case, despite a high pseudo total soil contamination, As, Cu and Pb concentrations in rosemary leaves were below international regulation levels concerning ingestion of medicinal herbs (no values available for Sb and Zn). MM concentrations in herbal teas were also under drinking water regulation. This study highlighted that health risks related to ingestion of contaminated rosemary, may be limited since ADI for As, Cu, Pb and Sb will require high quantities to be reached (from 50 g to almost 1 kg per day for an adult). As analyses were done only on washed plant parts and since this study took place in the second higher French peri-urban area, it could be interesting, in future studies, to investigate the possible contamination provided by dust due to aeolian transport. However, since MM are known not to be transferred during steam distillation, using contaminated rosemary for essential oil production could be a recovering opportunity. Moreover, essential oils' composition from rosemary individuals growing at the site contaminated by MM seemed to exhibit an enhancement of antioxidant compounds biosynthesis. Further studies will be necessary to confirm if these moderate variations of essential oil composition have an impact on its global antioxidant properties. With regard to the question of the treatment of hydrodistillation byproducts (water and residual biomass) containing MM, our results showed no accumulation of MM in edible parts of rosemary. Another perspective is to use rosemary for a phytostabilization process. Indeed, a previous study showed its ability to accumulate MM in the root parts and reduce MM phytodisponibility in soil (Madejon et al., 2009). Thus, speciation of contaminants, phytoavailability and mobility of MM in soil and in rhizosphere need to be investigated to evaluate rosemary potential for phytostabilization in this context.

Conflict of Interest

The authors declare that there are no conflict of interest.

Acknowledgments

This study was financially supported by the Agence Nationale de la Recherche, ANR CES Marséco program (2008 CESA 018) and by the National Innovative Cluster on Risks Management. Many thanks to Botlik L, Cézanne F, Ifrah C, Vinviollet D for their help to prepare the rosemary essential oils, to Bruel R for herbal tea preparation, to Vassalo L and Demelas C for their help for ICP-AES analyses, to Greff S. for GC-MS analyses and to Dumas PJ for the map. The authors thank the three anonymous reviewers for their constructive comments that improved the manuscript.

References

- Abdul-Wahab O, El-Rjoob AWO, Massadeh AM, Omari MN. Evaluation of Pb, Cu, Zn, Cd, Ni and Fe levels in *Rosmarinus officinalis* Labiatae (rosemary) medicinal plant and soils in selected zones in Jordan. *Environ Monit Assess* 2008;140:61–8.
- Adams RP. Identification of essential oils by gas chromatography quadrupole mass spectroscopy. Carol Stream, USA: Allured Publishing Corporation; 2001.
- Al-Sereitia MR, Abu-Amerb KM, Sena P. Pharmacology of rosemary (*Rosmarinus officinalis* linn.) and its therapeutic potentials. *Indian J Exp Biol* 1999;37:124–31.
- Angioni A, Barra A, Cereti E, Barile D, Coisson JD, Arlorio M, et al. Chemical composition, plant genetic differences, antimicrobial and antifungal activity investigation of the essential oil of *Rosmarinus officinalis* L. *J Agric Food Chem* 2004;52:3530–5.
- Apak A, Güçlü K, Demirata B, Özyürek M, Celik SE, Bektasoglu B, et al. Comparative evaluation of various total antioxidant capacity assays applied to phenolic compounds with the CUPRAD assay. *Molecules* 2007;12:1496–547.
- Aspadjan S, Celik G, Taskesen S, Güçer S. Arsenic, cadmium and lead in medicinal herbs and their fractionation. *Food Chem Toxicol* 2008;46:2871–5.
- Baars AJ, Theelen RMC, Janssen PJCM, Hesse JM, van Apeldoorn ME, Meijerink MCM, et al. Re-evaluation of human-toxicological maximum permissible risk levels RIVM, Rijnsinstituut voor volksgezondheid en milieu. Report 711 701 025; 2001.
- Baker AJM. Accumulators and excluders—strategies in the response of plants to heavy metals. *J Plant Nutr* 1981;3:643–54.
- Basol : basol.ecologie.gouv.fr, sheet n° 13.0098, update 13/03/2006.
- Ben Sghaier M, Skandrani I, Nasr N, Dijoux France MG, Chekir-Ghedira L, Ghedira K. Flavonoids and sesquiterpenes from *Teucrium ramosissimum* promote antiproliferation of human cancer cells and enhance antioxidant activity: a structure-activity relationship study. *Environ Toxicol Pharmacol* 2011;32:336–48.
- Berlin K, Gerhardsson L, Börjesson J, Lindh E, Lundström N, Schütz A, et al. Lead intoxication caused by skeletal disease. *Scand J Work Environ Health* 1995;21(4):296–300.
- Boskou D. Olive oil, chemistry and technology. 2nd ed. AOCS Publishing; 2006.
- Boutekedjiret C, Bentahar F, Belabbes R, Bessiere JM. Extraction of rosemary essential oil by steam distillation and hydrodistillation. *Flavour Fragr J* 2003;18:481–4.
- Cala V, Cases MA, Walter I. Biomass production and heavy metal content of *Rosmarinus officinalis* grown on organic waste-amended soil. *J Arid Environ* 2005;62:401–12.
- Cheung STJ. Anti-proliferative and antioxidant properties of rosemary *Rosmarinus officinalis*. *Oncol Rep* 2007;17:1525–31.
- Chojnacka K, Chojnacki A, Gorecka H, Gorecki H. Bioavailability of heavy metals from polluted soils to plants. *Sci Total Environ* 2005;337:175–82.
- Creclius EA. Changes in the chemical speciation of arsenic following ingestion by man. *Environ Health Perspect* 1977;19:147–50.
- Croteau R, Kutchan TM, Lewis NG. Chapter 24, products secondary metabolites. In: Buchanan B, Gruissem W, Jones R, editors. *Natural biochemistry and molecular biology of plants*. American Society of Plant Physiologists; 2000. p. 1250–318.
- Davis EM, Croteau R. Cyclization enzymes in the biosynthesis of monoterpenes, sesquiterpenes and diterpenes. *Top Curr Chem* 2000;209:53–94.
- Del Bano MJ, Lorente J, Castillo J, Benavente-Garcia O, Del Rio JA, Ortuno A, et al. Phenolic diterpenes, flavones, and rosmarinic acid distribution during the development of leaves, flowers, stems and roots of *Rosmarinus officinalis*. Antioxidant activity. *J Agric Food Chem* 2003;51:4247–53.
- Domiguez MT, Maranon T, Murillo JM, Schulin R, Robinson BH. Trace element accumulation in woody plants of the Guadamar Valley, SW Spain: a large-scale phytomanagement case study. *Environ Pollut* 2008;152:50–9.
- Dray S, Chessel D, Thioulouse J. Co-inertia analysis and the linking of the ecological data tables. *Ecology* 2003;84:3078–89.
- Ei-Kherbawy M, Angle JS, Heggo A, Chaney RL. Soil pH, rhizobia, and vesicular-arbuscular mycorrhizae inoculation effects on growth and heavy metal uptake of alfalfa (*Medicago sativa* L.). *Biol Fertil Soils* 1989;8:61–5.
- FAO/WHO. Food standards programme, codex alimentarius commission. Thirty-first sessions, International Conference Centre Geneva Geneva, Switzerland, 30 June – 4 July 2008 31th session – Alinorm 08/31/20; 2008.
- Fernández-Ugalde O, Virto I, Barré P, Gartzia-Bengoetxea N, Enrique A, Imaz MJ, et al. Effect of carbonates on the hierarchical model for aggregation in calcareous semi-arid Mediterranean soils. *Geoderma* 2011;164:203–14.
- Finch H. Comparison of the performance of nonparametric and parametric MANOVA test statistics when assumptions are violated. *Method Eur J Res Methods Behav Soc Sci* 2005;1(1):27–38.
- Heo M, Gabriel KR. A permutation test of association between configurations by means of the RV coefficient. *Commun Stat Simul* 1997;27:843–56.
- Herbal drugs monograph 1433. Pharmeuropa 2008;20(2):302–3.
- INERIS-Institut National de l'Environnement Industriel et des Risques (France). Fiche de données toxicologiques et environnementales du Plomb. Version 2; 2003.
- INERIS-Institut National de l'Environnement Industriel et des Risques (France). Fiche de données toxicologiques et environnementales du Cuivre. Version 1; 2005a.
- INERIS-Institut National de l'Environnement Industriel et des Risques (France). Fiche de données toxicologiques et environnementales du Zinc. Version 2; 2005b.
- INERIS-Institut National de l'Environnement Industriel et des Risques (France). Fiche de données toxicologiques et environnementales de l'Arsenic. Version 4; 2010.
- Jennings W, Shibamoto T. Qualitative analysis of flavor and fragrance volatiles by glass capillary gas chromatography. New York: Academic Press; 1980.
- Kaloustian J, Pauli AM, Pastor J. Evolution of camphor and other components in the essential oils of two labiate species during the biological cycle. *Analisis* 2000;28:308–15.
- Komnitsas K, Modis K. Soil risk assessment of As and Zn contamination in a coal mining region using geostatistics. *Sci Total Environ* 2006;371:190–6.
- Komnitsas K, Modis K. Geostatistical risk estimation at waste disposal sites in the presence of hot spots. *J Hazard Mater* 2009;164:1185–90.
- Laffont-Schwob I, Dumas PJ, Pricop A, Rabier J, Miché L, Affre L, et al. Insights on metal-tolerance and symbionts of the rare species *Astragalus tragacantha* aiming at phytostabilization of polluted soils and plant conservation. *Ecol Mediterr* 2011;37(special issue 2):57–62.
- Lasalle JL. Présence de plomb et d'arsenic sur le littoral sud de Marseille : une étude de santé (juillet 2005). INVS report; 2007.
- Lopez-Munoz F, Alamo C, Garcia-Garcia P. The herbs that have the property of healing: the phytotherapy in Don Quixote. *J Ethnopharmacol* 2006;106:429–41.
- Lotmani B, Fatarna L, Berkani A, Rabier J, Prudent P, Laffont-Schwob I. Selection of Algerian populations of the Mediterranean saltbush, *Atriplex halimus*, tolerant to high concentrations of lead, zinc and copper for phytostabilization of heavy metal-contaminated soils. *Eur J Plant Sci Biotechnol* 2011;5(special issue 2):20–6.
- Madejon P, Burgos P, Cabrera F, Madejon E. Phytostabilization of amended soils polluted with trace elements using the Mediterranean shrub: *Rosmarinus officinalis*. *Int J Phytorem* 2009;1:542–57.
- Manach C, Scalbert A, Morand C, Remesy C, Jimenez L. Polyphenols: food sources and bioavailability. *Am J Clin Nutr* 2004;79:727–47.
- Manly BFJ. Randomization, bootstrapping, and Monte Carlo methods in biology. 2nd ed. London: Chapman & Hall; 1997.
- Nath R, Pavur R. A new statistic in the one way multivariate analysis of variance. *Comput Stat Data Anal* 1985;2:297–315.
- Oomen AG, Tolls J, Sips AJAM, Groten JP. *In vitro* intestinal lead uptake and transport in relation to speciation. *Arch Environ Contam Toxicol* 2003;44:116–24.
- Ormeno E, Baldy V, Ballini C, Fernandez C. Production and diversity of volatile terpenes from plant on calcareous and siliceous soils: effect of soil nutrients. *J Chem Ecol* 2008;34:1219–29.
- Palero-Fernandez FJ, Martin-Izard A. Trace element contents in galena and sphalerite from ore deposits of the Alcudia Valley mineral field (Eastern Sierra Morena, Spain). *J Geochem Explor* 2005;86:1–25.
- Pilon-Smits E. Phytoremediation. *Annu Rev Plant Biol* 2005;56:15–39.
- Pounds JG, Long GJ, Rosen JF. Cellular and molecular toxicity of lead in bone. *Environ Health Perspect* 1991;91:17–32.
- Quevauviller P. Operationally defined extraction procedures for soil and sediment analysis I. Standardization. *Trends Anal Chem* 1998;17:289–98.
- Quevauviller P, Rauret G, Ure A, Bacon J, Muntau H. Report EUR 17127 EN. Brussels: European Commission; 1997.
- Rabier J, Laffont-Schwob I, Bouraïma-Madjèdi S, Léon V, Prudent P, Viano J, et al. Characterization of metal tolerance and accumulation in *Grevilla exul var exul*. *Int J Phytorem* 2007;9:419–35.
- Robert P, Escoufier Y. A unifying tool for linear multivariate statistical methods: the RV-coefficient. *Appl Stat* 1976;25(3):257–65.
- Schwob I, Bessiere JM, Masotti V, Viano J. Changes in essential oil composition in Saint John's wort (*Hypericum perforatum* L.) aerial parts during the phenological cycle. *Biochem Syst Ecol* 2004;32(8):735–45.
- Sekeroglu N, Ozkutlu F, Kara M, Ozguven M. Determination of cadmium and selected micronutrients in commonly used and traded medicinal plants in Turkey. *J Sci Food Agric* 2008;88:86–90.
- Sullivan C. Searching for nineteenth-century Florida water bottles. *Hist Archaeol* 1994;28:78–98.
- Sundar S, Chakravarty J. Antimony toxicity. *Int J Environ Res Public Health* 2010;7:4267–77.
- Testiati E, Parinet J, Massiani C, Laffont-Schwob I, Rabier J, Pfeifer HR, et al. Trace metal and metalloids contamination level in soils and two native plant species of a former industrial site: evaluation of the phytostabilization potential. *J Hazard Mater* 2013;248–249:131–41.
- Toda S. Polyphenol content and antioxidant effects in herb teas. *Chin Med* 2011;2:29–31.
- Todeschini V, Lingua G, D'Agostino G, Carniato F, Rocciotiello E, Berta G. Effect of high zinc concentration on polar leaves: a morphological and biochemical study. *Environ Exp Bot* 2011;71:50–6.
- World Health Organization (WHO). Guidelines for drinking-water quality First addendum to 3rd ed.; 2006 [Geneva].

- World Health Organization (WHO). WHO guideline for assessing quality of herbal medicines with reference to contaminants and residue; 2007 [Geneva].
- Yadav SK. Heavy metals toxicity in plants: an overview on the role of glutathione and phytochelatin in heavy metals stress tolerance of plants. *S Afr J Bot* 2010;76: 167–79.
- Yaylali-Abanuz G. Heavy metal contamination of surface soil around Gebze industrial area, Turkey. *Microchem J* 2011;99:82–92.
- Yenilmez F, Kuter N, Emil MK, Aksoy A. Evaluation of pollution levels at an abandoned coal mine site in Turkey with the aid of GIS. *Int J Coal Geol* 2011;86(1):12–9.
- Zaouli Y, Messouad C, Boussaid M. Genetic diversity of wild populations of *Rosmarinus officinalis* L. (Lamiaceae) in Tunisia. *Ecol Mediterr* 2003;29:199–216.
- Zheljazkov VD, Craker LE, Xing B. Effects of Cd, Pb, and Cu on growth and essential oil contents in dill, peppermint, and basil. *Environ Exp Bot* 2006;58:9–16.
- Zheljazkov VD, Craker LE, Xing B, Nielsen NE, Wilcox A. Aromatic plant production on metal contaminated soils. *Sci Total Environ* 2008a;395:51–62.
- Zheljazkov VD, Jeliaskova EA, Kovacheva N, Dzhurmanski A. Metal uptake by medicinal plant species grown in soils contaminated by a smelter. *Environ Exp Bot* 2008b;64: 207–16.