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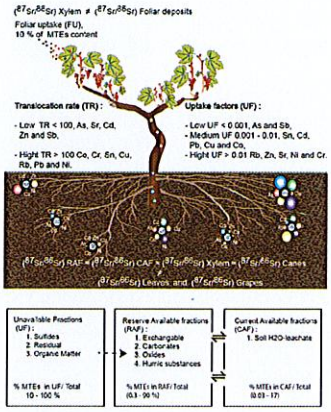
HIGHLIGHTS

- Sr isotopes are a suitable tracer for MTEs and As transfer in grapevine.
- Three MTEs and As reservoirs were identified in the studied soil.
- The highest MTEs and As concentrations were observed in leaves.
- Foliar uptake contributes up to 10% of the MTEs in leaves.
- The chronic As exposure is of great concern in the mining areas.

GRAPHICAL ABSTRACT



ABSTRACT

The transfer of metal and metalloid trace elements (MTEs) from contaminated soil to grapevines is a major issue for grape consumption and for the associated health risks. Based on an isotopic approach, we shed light on the concept of MTE bioavailability. The bioavailable fractions are identified by using the Sr-isotope ratio as a proxy for MTEs. This allows us to differentiate three soil reservoirs: the 'current available fraction' in soil water, the 'reserve available fraction' stored in mineral phases of the soil fractions, and the 'non-available fraction'. The reserve available fraction, representing 10 to 60% of bulk soil depending on the MTE, includes the exchangeable, carbonates, humic substance and oxides fractions. The $^{87}Sr/^{86}Sr$ isotopic signatures of grape berries and vine leaves show an additional source of MTEs, which is imported by foliar uptake and can contribute up to 10% of the MTEs in leaves. In addition, root-uptake and translocation rates show high accumulation rates of Co, Sn and Cu, and low ones for As, Sb, Zn and Cd. A daily intake between 1 and 3 kg of (dry grapes) would reach the benchmark dose level for a 0.5% ($BMDL_{0.5}$). While such a daily intake of grapes is unreasonable, consumption of other local vegetables and fruit would contribute to the daily intake. Hence, a chronic arsenic exposure is of great concern for human health in mining areas. We outline the importance of geochemical tracers, such as Sr isotopes, when determining the transfer and translocation of MTEs in plants. Our method presents a high-precision evaluation of the bioavailability and bioaccumulation of MTEs, and a better understanding of these processes in plants, thus leading to a better assessment of the environmental risk on human health.

Keywords:
Sr isotopes
MTEs
Arsenic
Bioavailability
Past mining area
Environmental risk
Grapevine

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1. Introduction

The composition of plants reflects the environment in which they grow (e.g. Kabata-Pendias, 2001, 2004, 2010), andbioavailability refers to the available amount of elements and their ability to be incorporated into biota (e.g. Kim et al., 2015; Olaniran et al., 2013; Rieuwerts et al., 1998). Up to now, the bioavailable fraction was considered to be associated with the soil exchangeable fraction and/or with carbonates (e.g. Hartman and Richards, 2014; Maurer et al., 2012; Song et al., 2014).

Bioaccumulation of metal and metalloid trace elements (MTEs) is a worldwide environmental issue, as it can adversely affect ecosystems and human health. This process will be amplified near mining areas, as the bioavailable fraction of MTEs is increased with respect to the pristine geochemical baseline (PGB) as provided by the INRA Infosol database (French National Institute for Agricultural Research). Its evaluation should provide valuable information regarding the risk of contaminant transfer and accumulation in the food chain (Kabata-Pendias, 2001). The bioavailable fraction is usually determined by single-step extraction on bulk soil, using buffered salts such as ammonium acetate (NH_4OAc) (Bertoldi et al., 2013; Cary et al., 2015; Song et al., 2014), NH_4NO_3 (Durante et al., 2016), or chelating agents such as diethylenetriaminepentaacetic acid–triethanolamine (DTPA–TEA) (Censi et al., 2014; Kabata-Pendias, 2004; Leita et al., 1998; Lindsay and Norvell, 1978; Vázquez Vázquez et al., 2016).

However, the calculated bioaccumulation index based on MTE concentration in soil, will differ significantly according to the method used for determining the bioavailable MTE fraction (Amorós et al., 2013; Bertoldi et al., 2013; Kabata-Pendias, 2010). Hence, this is a major issue when evaluating the bioaccumulation and transfer factors in vineyards; high-precision isotopic techniques thus may be a valuable tool for the tracking of MTE transfer and bioaccumulation in grapevines.

Hereafter, we propose a novel approach for identifying the bioavailable phase in all identifiable soil fractions (exchangeable, carbonates, Fe-Mn oxides, organic matter, humic substances, sulphides and residual), which is based on Sr-isotope signatures. To this end, we determined the Sr-isotope signatures of soil fractions obtained from sequential extraction, together with multi-element analyses of MTEs. Then, the obtained isotopic signatures and MTE concentrations of the different soil fractions were compared to those of plant tissues.

Sr isotopes are known to be a conservative tracer during nutrient uptake by plants (Capo et al., 1998). As the geochemical behaviour of Sr is quite similar to that of Ca in most natural environments and during most biological processes (Capo et al., 1998), it may be used as a proxy for Ca in plants.

On this basis, Sr isotopes are widely used in many fields, including in tracing the geographical origin of agricultural products (Bataille and Bowen, 2012; Durante et al., 2013; Gremaud et al., 2004; Kelly et al., 2005; Marchionni et al., 2013) and in archeological studies (Beard and Johnson, 2000; Degryse et al., 2010; Montgomery et al., 2007; Muller, 2003). Song et al. (2015) showed that the $^{87}Sr/^{86}Sr$ ratios of rice and hot-pepper plants were in agreement with those of water and of the exchangeable soil fraction as determined with NH_4OAc. Durante et al. (2016) showed the power of isotopic tools as geographical tracers, for tracking the origin of wine and for the processes controlling such traceability.

For this study, grapevines (*Vitis vinifera*) were selected as a case study model because:

1) They are relatively tolerant to metallic contamination, notably to arsenic;
2) They constitute a worldwide type of agriculture, covering over 800,000 ha in France alone; and
3) Their consumption as wine or table grapes may impact human health.

We focused on MTEs of major interest in bioavailability and risk assessment, including As, Cd, Cr, Cu, Ni, Sb, Sn and Pb that are considered as potentially hazardous metals in the environment (McKinney and Rogers, 1992). In order to quantify MTE distribution and bioaccumulation in grapevines, we analysed xylem sap, canes, leaves, and berries, in addition to chemical leachates of the vineyard soil.

Thus, the objectives of this study were: 1) Assess the bioavailable phases in soil for plant uptake using Sr isotopes, 2) Define the bioaccumulation and translocation factors in the soil-grapevine system, and 3) Demonstrate the influence of foliar uptake on MTE bioaccumulation.

Two observation sites (A and B) occupied by vineyards (Khaska et al., 2018) were selected near the Salsigne mining district in the Orbiel Valley (southern France). Both sites are affected by past mining activity and show high concentrations of As. See (Khaska et al., 2015a) for a historical, geological and general overview of the studied sites.

2. Materials and methods

2.1. Sampling, preparation and mineralization of grapevine tissues

Vineyard soil and vine samples were collected from two sites, located near the reclaimed gold-mining area of Salsigne in southern France (Fig. S1).

At site A, agricultural practices are fully natural and no organic nor inorganic treatments are applied. At site B, synthetic organic pesticides are applied to the vines. The sampled grape varieties are Carignan and Merlot at sites A and B, respectively. Grapevine tissues (leaves, canes, berries, and xylem sap), and soil samples were collected from both sites. Five hundred millilitres of xylem sap (reflecting the uptaken water, nutrients and soil contaminants) were collected during the first 3 days of bleeding sap. Leaves, grapes, and canes were collected from both sites in late summer, and frozen at −20 °C until analysed. For each sampling site, two hundred berries from ten bunches were randomly selected. All samples were cleaned manually and washed twice with deionized water in an ultrasonic bath, before washing with a 0.2 M solution of nitric acid (or 1% HNO_3) in order to remove any remaining atmospheric-dust deposits. Samples were then dried at 60 °C and ground for homogenization. About 400 mg of each tissue and 5 mL of xylem sap were mineralized by assisted microwave extraction (AME) using a Milestone Ethos Microwave in PFA vessels at the CHROME-team GIS laboratory of the University of Nîmes, using HNO_3, H_2O_2 reagents (see Table S1 in Supporting information). In parallel, raw xylem sap was also analysed after filtration at 0.22 µm and acidified to pH ≈ 2. Results are compared in the Supporting information (Fig. S2) and are fairly coherent.

2.2. Selective sequential extraction from soil samples

Soil samples were collected around the grapevine trunk. They were then dried, sieved at <1 mm and homogenized before analysis. For each site, four sub-samples were collected from the surface horizon (0–40 cm). Five hundred grams from each soil sub-sample were pooled and vigorously homogenized to obtain a composite soil sample.

One gram of composite soil was used for selective sequential extraction according to the procedures modified from Campanella et al. (1995) and Tessier et al. (1979) (Table S2, Supporting information). In addition, to evaluate the potential for mobilization of Sr and MTEs during a short water-soil contact—as would occur in the field during infiltration of rainwater, irrigation water, or during flooding events—, a leaching experiment was carried out using 1 g of composite soil in 10 mL deionized water under constant agitation for 24 h (Table S2). Finally, 1 g of composite soil was totally mineralized by AME to determine the total concentration of MTEs and Sr in the soil, and to assess the recovery of the sequential extraction (Table S2). For comparison with the sequential extraction method, 1 g of soil was leached with NH_4OAc and 1 g with DTPA/TEA/$MgCl_2$.

The concentration of each fraction is expressed in concentration with respect to the mass of the bulk sample. The overall recovery rate

of the sequential extraction, modified from Tessier et al. (1979) is calculated as the sum of MTEs associated with each fraction over the total concentration measured on bulk samples. The recovery rates vary between 70% and 120% for MTEs (except Pb), 65% and 80% for Sr, and between 90% and 110% for As for sites A and B, respectively.

The physico-chemical parameters of the soil samples (texture, pH, carbonate content, organic-matter content, cationic-exchange capacity, and oxide content) were determined at the Alsacian Society for the Development and Study of Fertility (SADEF), in compliance with, respectively, the NF ISO 11464, NFX 31-107, NF ISO 14 235, NFX 31-130, NFX 31-121 and NFX 31-108 standards of the French accreditation organization COFRAC. The results are presented in Table S3 (supporting information).

2.3. Analytical methods

All soil-leachate and vine-tissue samples were evaporated on a hot plate before conditioning in 50 mL of HNO_3 (2% v/v). As, Cd, Cr, Cu, Ni, Zn, Co, Sb, Sn, Pb, Sr, and Rb concentrations were determined by inductively coupled plasma mass spectrometry (ICP-MS) on an Agilent 7700x Quadrupole ICP-MS at the AETE-ISO regional facility (OSU OREME, University of Montpellier, France). Analytical uncertainty is around 5%.

Sr-isotope analyses were determined by thermal ionization mass spectrometry (TIMS) at the CHROME laboratory of the University of Nîmes. The analytical procedures of Sr isotope separation and analysis are described in (Khaska et al., 2015b; Pin et al., 1994; Sassine et al., 2015).

3. Results

3.1. Vineyard soil MTEs

Among the assayed MTEs, only arsenic concentrations exceeded the pedo-geochemical baseline (PGB) concentration of 31 $mg \cdot kg^{-1}$, as defined in uncontaminated soil near the study area (INRA Infosol database). Arsenic concentrations in soil were 134 and 87 $mg \cdot kg^{-1}$ for sites A and B, respectively (Table S4), close to those observed in mining areas in Italy by Bertoldi et al. (2013). These values are 3 to 4 times higher than the local PGB, and 9 to 13 times higher than in uncontaminated soils, where As contents are typically below 10 $mg \cdot kg^{-1}$ (Adriano, 1986). According to the United States Environmental Protection Agency (US. EPA, 1990), such As concentration levels in soil reach the trigger level for remediation at 100 $mg \cdot kg^{-1}$. The presence of As in soil is mainly due to arsenopyrite, which was the predominant ore in the former Salsigne mine. Cu, Zn, Cr, Ni and Pb show concentrations in the range of 24 to 203 $mg \cdot kg^{-1}$, while the concentrations of Co, Cd, Sn and Sb are much lower, ranging from 0.6 to 3 $mg \cdot kg^{-1}$. The influence of past mining activities on the PGB in terms of MTE concentrations in the study area, was earlier described in Khaska et al. (2018).

The partitioning pattern of MTEs in soils as defined by the selective extraction procedure, shows that As, Cr, Cu, Rb, Sb, Sn and Cd are similarly distributed in both soils, while Zn, Pb, Ni, and Co differ slightly (Fig. 1). As expected, the labile Sr shows higher concentrations when associated with the carbonate fraction in site A, while on site B most of the labile Sr is associated with the exchangeable fraction. These results are coherent with the low $CaCO_3$ content of the soil on site B, which at 8% is less than half that for site A (19%).

Concerning the one-step extraction procedure, a good correspondence is observed for MTE distribution in the DTPA/TEA leachate versus that of NH_4 OAc (Fig. S3). We also observe a good correlation between the sum of the carbonate and exchangeable fractions extraction, and that of the DTPA/TEA and NH_4OAc leachates.

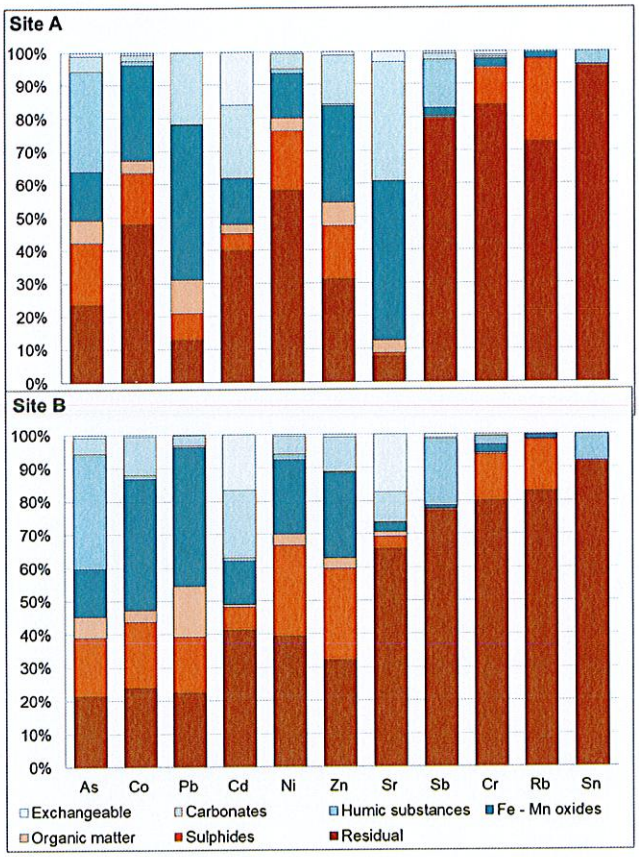

Fig. 1. Distribution of As and MTEs in the different soil fractions in both sites.

3.2. Rapevine tissue

The pattern of MTE translocation from xylem sap to canes, leaves and grapes shows a bioaccumulation in leaves and a retention from leaves to grapes. Similar profiles were observed at both sites (Fig. S4). Compared to the xylem sap, the MTE concentrations are up to 90 times higher in canes and up to 370 times higher in leaves. In contrast, the concentrations of MTEs are up to 15 times lower in grape berries than in leaves. For arsenic for example, its concentrations in xylem sap are only a few $\mu g \cdot kg^{-1}$ dry weight (dw), while in leaves they reached up to 1282 and 400 $\mu g \cdot kg^{-1}$ dw and in grapes 83 and 38 $\mu g \cdot kg^{-1}$ dw, at sites A and B respectively. These results also fall within the range of concentrations measured in grapevine leaves and berries in mining areas in Italy (Bertoldi et al., 2011, 2013). Lower Cd, Sn and Sb concentrations were observed in the grapes, canes, leaves and xylem (Table S4).

Sr shows a similar pattern as MTEs (Fig. S4). Rb, showed the highest concentration in grapes, which is consistent with the fact that Rb behaves similarly to K, moving via the phloem into the grape and accumulating there after veraison (Bertoldi et al., 2011).

3.3. Vineyard-soil and grapevine $^{87}Sr/^{86}Sr$ ratios

The Sr composition of soil and vines is likely to be influenced by the $CaCO_3$ isotopic signature of the soil, and by agricultural treatment and pedo-genetic evolution. Among all solid soil fractions, the residual fractions (0.73141 and 0.72909 for sites A and B, respectively) and sulphide fractions (0.71388 and 0.71689 for sites A and B, respectively) yielded the most radiogenic Sr-isotope ratio (Fig. 2). Carbonate, Fe-Mn oxide, humic substance and exchangeable fractions all show similar signatures

to that of the soil H_2O-leachate (0.70879 and 0.71047 for sites A and B, respectively). The Sr signature of organic matter is similar to those phases at site A, but differs significantly at site B. This may be due to agricultural treatment input with a higher Sr isotopic signature of 0.71346.

Apart from the residual and sulphide fractions, the $^{87}Sr/^{86}Sr$ isotopic signatures of all studied soil fractions were significantly more radiogenic in site B (0.70991 to 0.71689) than in site A (0.70829 to 0.70879).

As far as the plants are concerned, the $^{87}Sr/^{86}Sr$ signatures of the xylem and cane tissues are similar to that of the labile fraction of vineyard soil obtained by H_2O-leachate (0.70879 for site A and 0.71047 for site B). However, the $^{87}Sr/^{86}Sr$ ratios of leaves and grape berries at both sites, (Table S4) differ significantly from those of the xylem sap or canes, suggesting the contribution of an additional Sr source in leaves and grapes (Fig. 3).

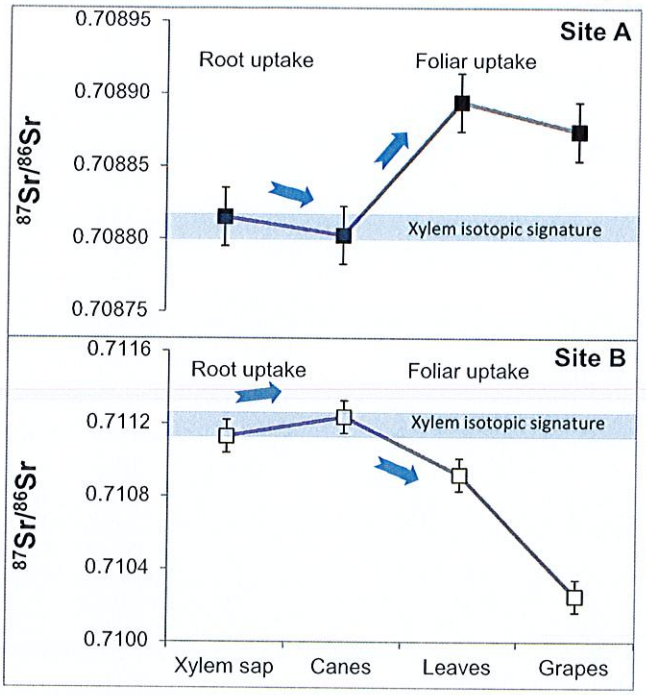

Fig. 3. Comparison of the $^{87}Sr/^{86}Sr$ ratios of the grapevine tissues, showing a significant difference between xylem sap, canes, leaves and grapes.

4. Discussion

4.1. Strontium as a tracer of the bioavailable fraction

Higher MTEs content in grapevine in site A than in site B may be explained by the difference of MTEs contents in soil where the sum of MTEs concentrations systematically being higher on site A than on site B. However, similar uptake pattern processes are observed at both sites A and B (Fig. 1).

In addition, the observed difference of the soil types, physical chemical characters but also the difference of grape varieties is all potential factors affecting uptake and translocation in the grapevine.

As mentioned before, the choice of Sr as a proxy for MTEs during transport in the soil/grapevine system was mainly based on its geochemical similarity with calcium, an essential nutrient for plant growth and development (Keller, 2015; Thomas et al., 1984). Hence, in plants, Sr can substitute for calcium in tissues (Hutchin and Vaughan, 1968; Queen et al., 1962; Thomas et al., 1984; White and Broadley, 2003); therefore, as Sr is relatively omnipresent and abundant in soil, water and plants, it constitutes a useful tracer.

The linear correlation between Sr concentrations and the sum of MTEs in grapevine tissues (Fig. 4) confirms that Sr and MTEs have a similar behaviour during nutrient uptake and translocation in the grapevine, as suggested in Section 3.2. This makes Sr into a suitable tracer of MTE transfer.

As shown in the $^{87}Sr/^{86}Sr$ vs. Sr diagram (Fig. 2), the similarity between Sr signatures of the exchangeable, humic-substance, carbonate and Fe-Mn-oxide-leachate fractions, and those of grapevine tissues (xylem sap, canes, leaves, and grapes) indicates a possible direct transfer of Sr from these four solid soil fractions to the grapevine. Consequently, we can assume that the bioavailable MTEs in soil come from those four phases. In addition, the isotopic signature of the H_2O-soil leachate both in composition and concentration is very close to that of the xylem, which suggests that the soil H_2O leachate represents the direct bioavailable fraction for plant uptake.

However, the significant contrast in $^{87}Sr/^{86}Sr$ ratios between on one hand, sulphides and the residual-fraction leachates, and on the other hand the grapevine tissues, suggests that MTEs associated with the former two fractions are unavailable for grapevine uptake. This is due to the slow dissolution kinetics of sulphides under the prevailing oxidation-reduction conditions of soil, which at the time of observation

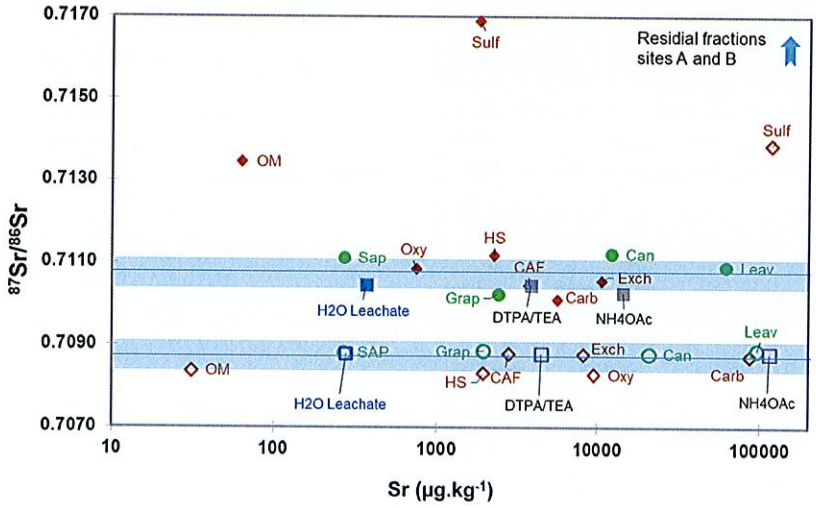

Fig. 2. Comparison of $^{87}Sr/^{86}Sr$ ratios of the soil fractions and Sr in grapevine tissues in sites A (open symbols) and B (closed symbols). Note the isotopic contrast between both sites, as well as the isotopic-signature contrast between the residual, sulphidic and organic-matter fractions and the other group. HS: humic substances; Carb: carbonates; Exch: exchangeable; Sulf: sulphides; OM: organic matter; CAF: current available fraction (in $\mu g \cdot kg^{-1}$) or H_2O soil leachate (in $\mu g \cdot L^{-1}$); Sap: xylem sap; Leav: leaves; Grap: grapes; Can: canes.

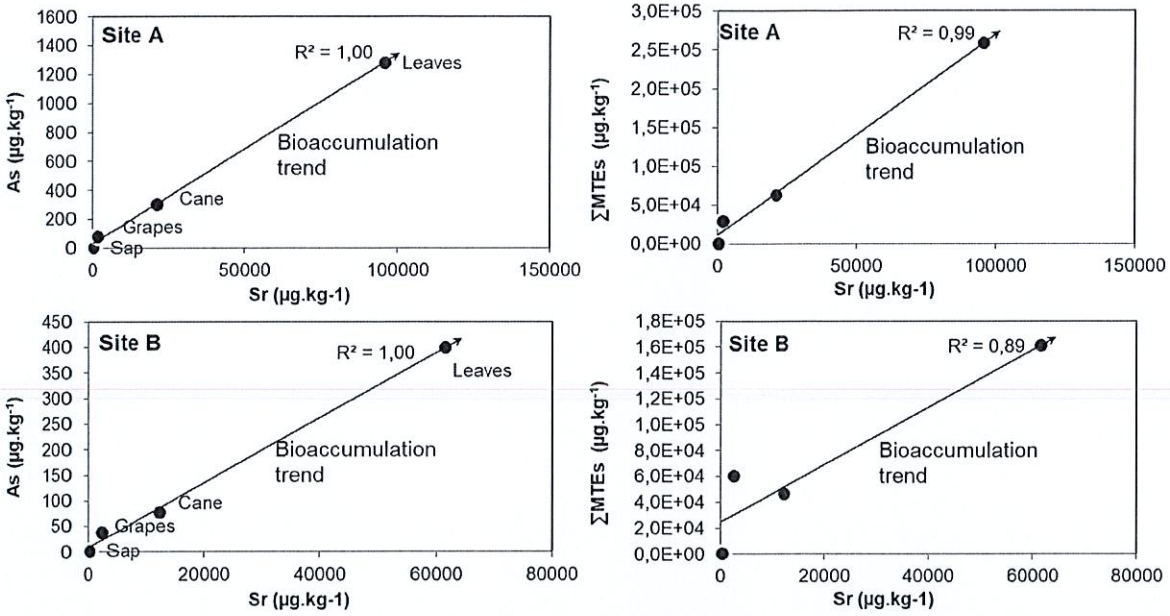

Fig. 4. Arsenic and the sum of MTE concentrations vs. Sr concentrations in grapevine tissues at sites A and B.

ranged between 15 to −20 mV for sites A and B, respectively, and the slow hydrolysis rate of silicate minerals in soil (White, 2003). Consequently, sulphide and silicate residual fractions are thus unlikely to contribute to the MTE content in grapevine tissues.

Similarly, the contrast between the $^{87}Sr/^{86}Sr$ ratios of organic matter and of vineyard tissues, at least at site B (Fig. 2), suggests that this fraction is unavailable for grapevine uptake.

Depending on the trace element, the overall bioavailable fraction of MTEs—including the exchangeable fraction, carbonates and Fe-Mn oxides—varies between 13% and 76% of the total amount of elements present in the bulk composition for site A, and between 12% and 63% of that for site B.

Rb, Cr, Sn, Ni and Sb are mainly present in the residual, organic-matter and sulphide fractions, while Co, Zn, Cd, As, Cu and Pb are more-or-less evenly distributed between the unavailable fraction, and the available fraction (the carbonate, exchangeable, oxide and humic-substance fractions).

4.2. Uptake of MTEs by the grapevine

Nevertheless, the bioavailable phases, as defined through the sequential leachate, are not directly available for absorption by roots, due to the chemical equilibrium kinetics between mineral phases and the soil solution. MTEs in the exchangeable fraction are taken up by cationic exchange, allowing easy uptake of metals and metalloids sorbed on the exposed surfaces of soil components and organic matter, including humic substances. Indeed, the root exudates contribute to MTE release from the exchangeable fraction, especially from the rhizosphere that extends to about 1 to 3 mm from the root surface into soil (Hinsinger, 2001; Thomas et al., 1984). Elements bound to carbonates are released by dissolution, a process that is susceptible to pH changes. Elements bound to Fe-Mn oxides can be released either by exchange or dissolution of oxides that are susceptible to oxidation-reduction conditions in the soil. The mobilization of MTEs and As from iron oxides has been shown by many authors (e.g. Khaska et al., 2018, or Palumbo-Roe et al., 2015). Once in solution, the elements are readily available for plant uptake. This method do not differentiate between amorphous and crystalized iron oxide phases, though (Pierce and Moore, 1982) showed that amorphous Fe-oxy-hydroxides show more reactive surfaces compared to crystalized iron oxides, subsequently, amorphous Fe-oxy-hydroxides may present a more important potential for MTEs and As bioavailability.

The uptake and incorporation of humic substance in plants was demonstrated based on ^{14}C-labeled (Vaughan et al., 1974; Vaughan and Linehan, 1976; Vaughan and MacDonald, 1976; Vaughan and Ord, 1981). It was shown that the low (<3500 Da) molecular-size fraction may easily reach the plasmalemma of higher plant cells, while the high-molecular size (>3500 Da) rather interacts with the cell wall (Nardi et al., 2002). Consequently, the MTEs associated with humic substance may also be absorbed by roots and taken up by tissue.

We consider that the sum of the exchangeable, carbonate, oxide and humic-substance fractions represents a 'reserve available fraction' (RAF), and that the soil solution in equilibrium with the RAF constitutes a 'current available fraction' (CAF). The CAF may be represented by the soil H_2O-leachate as mentioned above, its chemical composition being controlled by different chemical processes, such as ionic exchange, dissolution/precipitation, oxidation/reduction, and microbiological activity. In addition, anthropogenic factors, such as irrigation and the addition of fertilizers and other chemical products, contribute strongly to the interaction between CAF and RAF.

Hence, we suggest that, as roots take up nutrients and other elements from the soil solution, the sheer uptake factor (UF) can be calculated relative to the MTE concentrations in the CAF. This shows that the root uptake factor is independent of the unavailable soil fraction as well as of the chemical equilibrium kinetics involved in mobilizing the RAF into solution.

$$UF_{xylem/CAF} = \frac{C_{Xylem\ sap}}{C_{CAF}} \quad (1)$$

The obtained uptake factors between CAF and xylem vary widely between 0.0001 and 0.1, depending upon the MTEs. The observed $UF_{xylem/CAF}$ variations reflect the high selectivity of roots in uptaking MTEs from soil (Kabata-Pendias, 2010). The MTEs are ranked according to increasing uptake factors (Fig. 5). The $UF_{xylem/CAF}$ is compared to the $UF_{xylem/Bulk}$ and $UF_{xylem/RAF}$. Both sites, A and B, show very similar uptake patterns.

The uptake factors may be classified into three categories

- Low uptake ability with UF below 0.001, which includes Sb and As.
- Medium uptake ability with UF ranging from 0.001 and 0.01, including Sn, Cd, Pb, Cu, Ni and Co,
- High uptake ability with UF above 0.01, including Rb, Zn, Sr, Ni and Cr.

Compared to uptake factors calculated with respect to bulk-rock concentrations or to the RAF, the $UF_{xylem/CAF}$ is at least one order of magnitude higher. The overall MTE distribution remains roughly similar, though Pb shifted from the low- to the medium-uptake group, while As and Sb shifted downward, showing the lowest uptake factor and reflecting the variable solubility of the elements.

It is also noticeable that the calculated UF relative to the RAF, is higher than that calculated relative to DTPA or NH_4OAc, and a preferential MTE uptake differing significantly between these phases. Therefore, the bioaccumulation index may be under-estimated if the DTPA/TEA or NH_4OAc is considered as reference for the MTE amount in soil.

4.3. Translocation rate (TR) of MTEs in V. vinifera L. tissues

Once in the xylem, MTEs are transported to the different parts of the plants and may accumulate differently (Keller, 2015). Here we consider the translocation rate (TR) in the different plant parts with respect to the MTE concentrations in the xylem phase.

We calculated the TR of MTEs in leaves and grapes with respect to the CAF at sites A and B (Fig. 6). The TR of MTEs in grapes can be grouped into two categories:

- MTEs with a low TR, <100, including Sr, As, Cd, Zn and Sb;
- MTEs with a high TR, >100, including Co, Cr, Sn, Cu, Rb, Pb and Ni.

Based on these data, the MTEs in leaves can also be divided into two groups:

- MTEs with a low TR of 100 to 1000, including Pb, Sb, Cr, Sr, As, Cd and Ni;
- MTEs with a high TR, >1000, including Co, Cr, Cu and Pb.

The TR for Sr, As, Zn, Sb and Cd is ten times higher in leaves than in grapes, but for Cr, Pb, Ni, Zn, Sn and Cu, it is slightly less in leaves than in grapes. The translocation rate in leaves is around 100 to 1000 for most MTEs, except for Co that shows the highest translocation rate.

For Pb, Ni, Sn, Cr and Co, the TR is 100 times higher at site B than at site A.

At site A, grapes accumulate up to ten times less MTEs than leaves. A similar MTE distribution is shown at site B for low accumulation factor

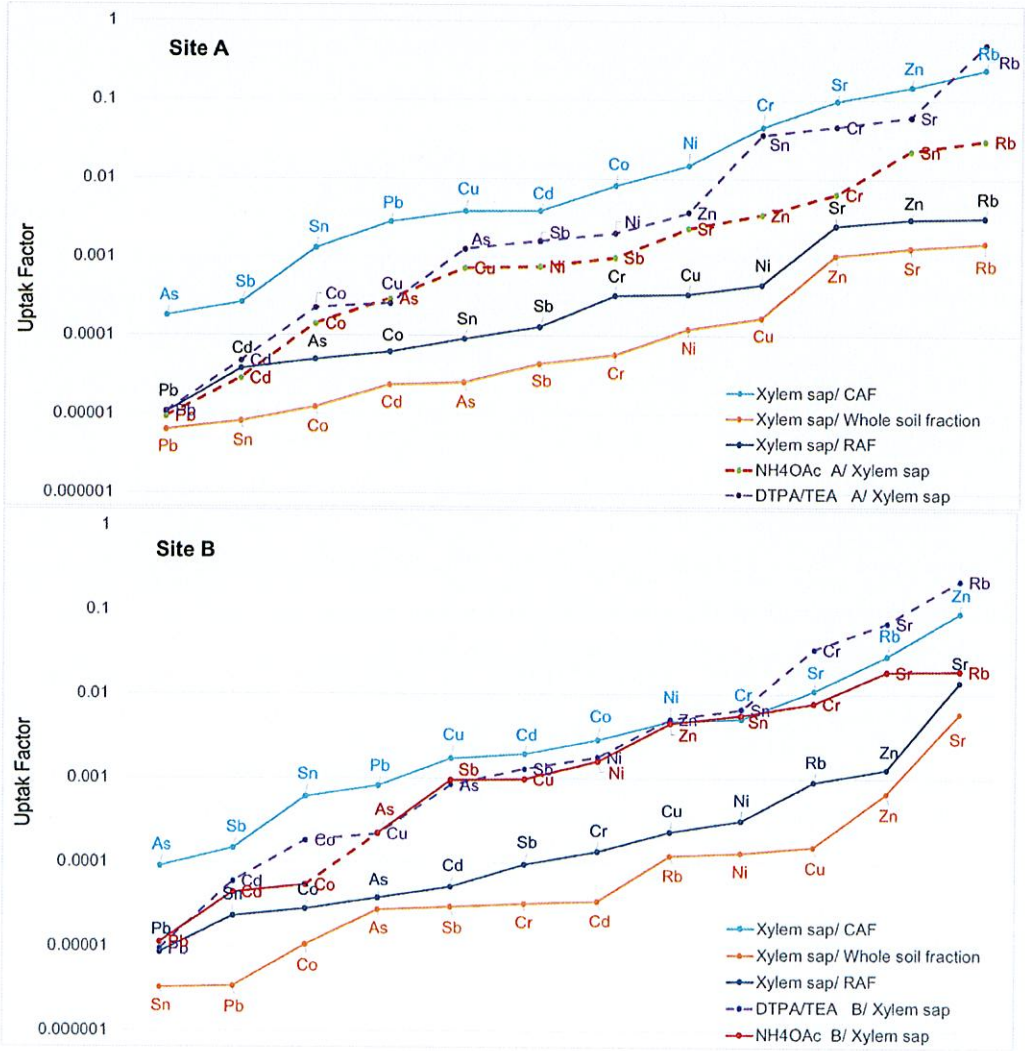

Fig. 5. The evolution of uptake factors for the different MTEs. These values were calculated as the ratio between the xylem and the CAF.

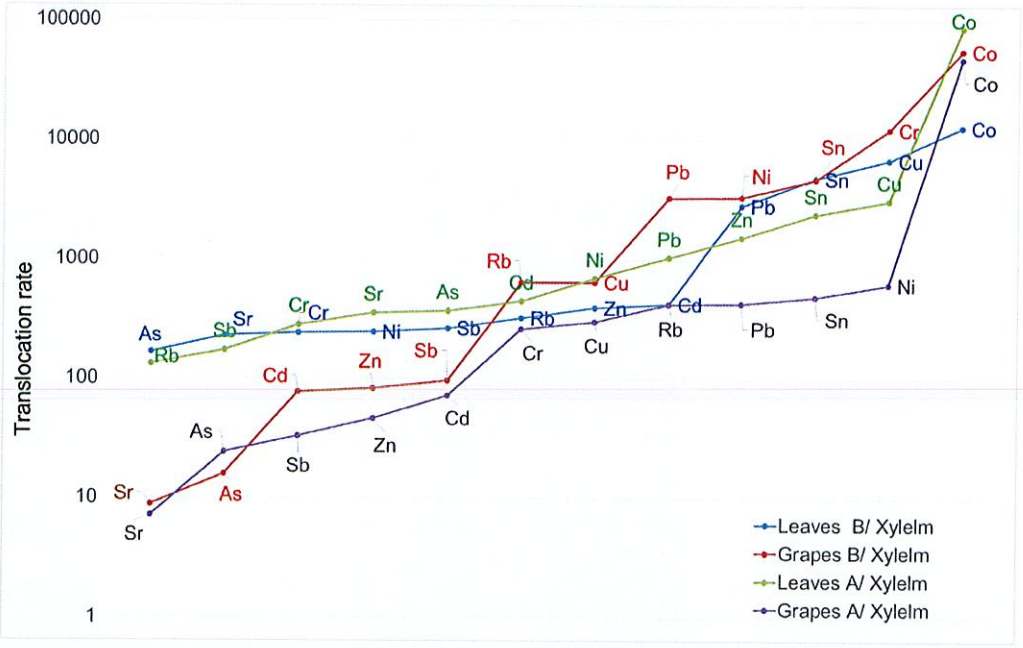

Fig. 6. Translocation rate of trace elements by grapevine from soils. Bioaccumulation index was calculated as the ratio of trace elements in grapevine compared to their concentration in the current available fraction (CAF).

MTEs as at site A, but for the higher accumulation factor MTEs the accumulation in grapes becomes 10-fold higher than in leaves. This might be related to chemical treatment of the vines at site B.

Overall, the bioaccumulation indexes of trace elements in the different grapevine tissues are related to different factors such as the bioavailability, the absorption by roots and/or leaves, and the translocation rate. Some of the trace elements are active, such as Zn, Cu and Ni that are bound to proteins and enzymes in plants, and as such participate in plant metabolism (Alagić et al., 2016; Keller, 2015). Others are passive such as Pb, As and Cd (nonmetabolic) and just accumulate in grapevine tissues and organs such as roots (e.g Angelova et al., 1999). At higher concentrations MTEs can eveal toxic to plants. However, according to previous studies on the tolerance and phytotoxicity of As and MTEs (Alagić et al., 2016; Carbonell-Barrachina et al., 1997; Juang et al., 2012; Pavlovkin et al., 2016; Tiecher et al., 2017), and considering the observed concentration of these elements at our site, there is no expected phytotoxic effect on the studied grapevines.

The evolution of MTE concentrations between the bioavailable soil fractions and grapevine parts is shown for sites A and B (Fig. 7).

4.4. Evaluation of foliar uptake and implication for the Sr signature

The relative enrichment of MTEs in leaves is directly related to the water cycle in the grapevine, but may also be influenced by direct foliar uptake. The stomats on the lower leaf surface play an important role in the exchange of gases (CO_2, O_2), food and water, to and from the leaf surfaces (Baldi et al., 2014; Lange et al., 1982). Exchange with the atmosphere involves the loss of water by transpiration and its absorption by leaf surfaces. MTEs deriving from atmospheric deposits (dust) will diffuse inside the leaf surfaces. The MTE absorption may be mobilized by rainwater, dew, or directly from fog (Boucher et al., 1995; Breshears et al., 2008).

Foliar water uptake has been largely demonstrated by many authors. Based on isotopic tracers (O, H), for instance, Limm et al. (2009) showed that foliar uptake contributes directly to increase the water content in leaves by 2–11%. In our study, $^{87}Sr/^{86}Sr$ ratios also revealed to be suitable tracers for tracking the implication of foliar uptake on the MTE budget in leaves. Any Sr from atmospheric deposits or anthropogenic sources may present a distinct $^{87}Sr/^{86}Sr$ signature compared to that of available soil fractions. The $^{87}Sr/^{86}Sr$ ratio of atmospheric deposits may depend on geological formations outcropping at the regional scale, and on aerosols, mining activities, and application of pesticides or other products used in agriculture for treating vines, such as $CuSO_4$. In mining areas, dust analyses show high concentrations of As, Sb, Cd, Co and Cr (around 400, 120, 29, 10 and 170 $g \cdot kg^{-1}$, respectively (ADEME, 2007)). The increase in $^{87}Sr/^{86}Sr$ ratios in grapes and leaves relative to xylem sap and canes at site A may be explained by the influence of radiogenic Sr through foliar uptake from the surrounding environment, characterized by a radiogenic Sr signature ranging from 0.7149 to 0.7226 and inherited from outcropping Paleozoic basement rocks (Khaska et al., 2015a, 2015b). At site B, however, the relatively depleted Sr signature in grapes and leaves compared to that of canes and xylem sap, may be due to the contribution of Sr from fungicides used in vine treatment. This depleted Sr signature is around 0.70916 ± 0.0002 (Khaska et al., 2013), relative to local atmospheric deposits.

The isotopic contrast between root uptake and foliar uptake is highly significant at site B (varying by 1 on the third decimal of the $^{87}Sr/^{86}Sr$ ratio), suggesting a non-negligible impact of fungicide applications. It is less significant at site A where no spraying occurs, varying by 1 on the fourth decimal $^{87}Sr/^{86}Sr$ ratio, showing less influence of local dust deposits. Based on appropriate mixing equations (Faure, 1986), between xylem and external Sr sources, such as fungicide or dust particles, the Sr amount required to shift the Sr-isotopic composition of leaves by 1 on the third decimal represents 90% from the Sr concentrations in xylem sap and about 10% from foliar uptake.

Contrasting with leaves and grapes, the isotopic Sr signature is dominated by the xylem sap signature according to the water cycle in plants. The isotopic implication of such water cycling is shown by the similar $^{87}Sr/^{86}Sr$ ratios of xylem sap and canes.

Finally, a conceptual scheme was drawn to illustrate the transfer of MTEs in the spoil-vineyards system (Fig. 8).

4.5. Environmental risk evaluation of grape consumption

Up to 2010, the United Nations Food and Agriculture Organization (FAO) and the World Health Organization (WHO) recommended a

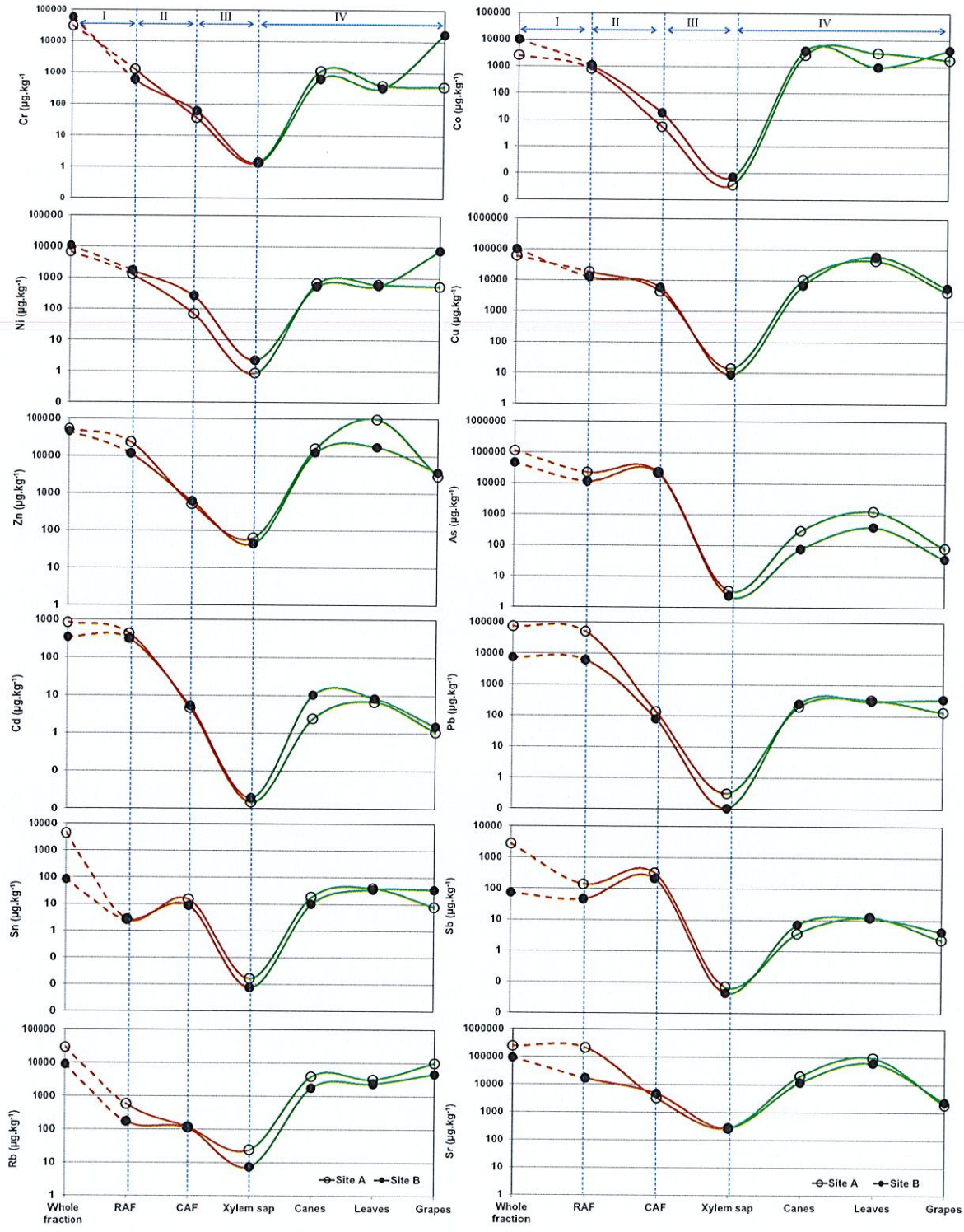

Fig. 7. Evolution of MTE concentrations between the bioavailable soil fractions and grapevine components. I presents a very low kinetic dissolution of unavailable soil fractions; II is the interaction between soil- and water-available fractions; III presents the pure roots uptake from CAF; IV is the MTEs translocation in grapevine.

provisional tolerable weekly arsenic intake of 15 µg/kg body weight (bw), or a daily intake of 2.1 µg/kg bw. However, in 2010 their review highlighted that the lowest calculated benchmark dose level for a 0.5% ($BMDL_{0.5}$) increased incidence of lung cancer above background, over an average of 11.5 years, was 3.0 µg/kg body weight per day. Though within the same order of magnitude as the newly estimated $BMDL_{0.5}$, the former guideline is no longer seen as appropriate. Considering this and an arsenic concentration of 40 to 80 µg/kg dw in grapes, a daily consumption of between 1 and 3 kg of dry grapes (or ~15 kg of fresh grape) would reach the $BMDL_{0.5}$. While such a daily grape intake is

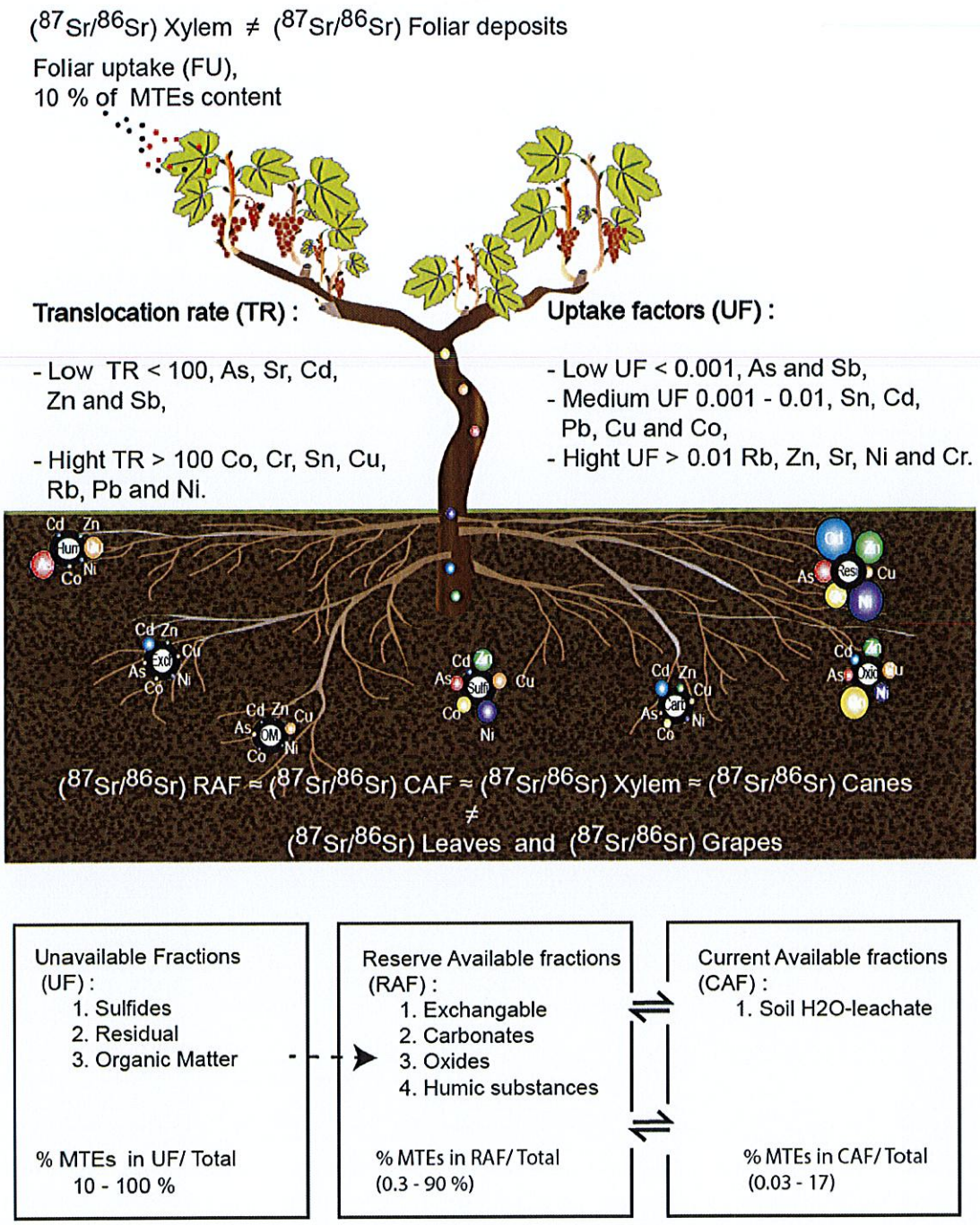

Fig. 8. Conceptual scheme of As and MTEs transfer and translocation in the soil-grapevine system based on $^{87}Sr/^{86}Sr$ signatures of the different compartments in this system.

unreasonable, consumption of other local vegetables and fruit would further contribute to the daily intake. Hence, a chronic arsenic exposure is of great concern for human health at our study sites, as well as in the former mining area where arsenic is persistent and omnipresent in the total environment, even one decade after mine closure.

Our results reinforce a regional statistical and epidemiological study carried out around the Salsigne mining area and funded by the French National Public Health Network, evaluating arsenic exposure and its health effects (Fréry et al., 2000). This study included 681 inhabitants from four villages around the former mining area. The main outcome was the identification at regional scale of a low-dose arsenic overexposure of the population, due to the consumption of local wine, fruits and vegetables, and contaminated groundwater. Moreover, higher arsenic levels were observed in flooded soil, similar to those of our studied sites A and B (Khaska et al., 2018). In view of the new $BMDL_{0.5}$, this epidemiological study should be revised.

5. Conclusions

The proposed approach contributes to complement previous and multiple methods as it relies on a detailed study of the different soil phase contribution allowing a precise description of the reservoir. We consider seven soil fractions corresponding to the principal identified fractions in soil, extending on initial approach which relies on five soil

fractions (Tessier et al., 1979). This comprehensive method allows validating the quantitative approach with satisfactory overall recoveries. In addition, the coupling of the isotopic approach with selective extraction allows to evaluate and to confirm the contribution of each soil phase to plant uptake. Comparison with the different methods by using one-step procedures leads to good matches with some specific steps of our procedure.

A good correlation was found between Sr and MTE concentrations in grapevine tissues, confirming the use of Sr for studying the MTE transfer process in this system. In our study, the Sr isotopes were a suitable tracer for tracking the uptake and translocation of As and MTEs in the soil-grapevine-leaf system. Using the Sr isotopes, we defined three soil reservoirs in the vineyard soils with respect to root uptake:

1) The 'reserve available fraction' (RAF) includes the exchangeable, carbonate and Fe-Mn-oxide fractions, as a "savings account";
2) The 'current available fraction' (CAF) in the soil H_2O-leachate is the "current account"; and
3) The unavailable fraction includes sulphidic and residual minerals.

The amount of bioavailable MTEs associated with these fractions varies significantly between MTEs, from 4% (for Sn) to 75% (for As), relative to the total MTE amount in bulk soil.

Strontium isotopic signatures in the vine leaves and grape berries are influenced by an atmospheric-dust fraction through a foliar uptake process. Up to 10% of MTEs in leaves, issued from this dust reservoir, was identified at the treated site B.

The $^{87}Sr/^{86}Sr$ signature of the dust fraction contrasts with that of the phyto-available phase of the soil, meaning that the $^{87}Sr/^{86}Sr$ signature may be different in the leaves and fruit of a plant. This has an implication for the reliability of using Sr as a tracer of the geographic origin of agricultural products.

A 'lower' uptake ability was observed for Sb and As, with a UF >0.01. The 'higher' UF was calculated for Zn, Ni, Cr, Sr and Rb. Finally, Sn, Cd, Pb, Cu and Co show intermediate uptake factors between 0.001 and 0.01.

Similarly, the translocation rate (TR) in grapes varies significantly between 10 and 7000 according to the MTE. The highest TRs were observed for Co, Sn, Cu and the lowest were for As, Sb, Zn and Cd. The TR for Sr, As, Zn, Sb and Cd is ten times higher in leaves than in grapes, but for Cr, Pb, Ni, Zn, Sn and Cu, it is slightly less in leaves than in grapes.

Our study outlines the importance of geochemical tracers, such as Sr isotopes, in the determination of pollutant transfer and translocation in plants. It presents a high-precision evaluation of the bioavailability and bioaccumulation of MTEs, and valuable data for a better understanding of these process in plants, and thus for a better assessment of the environmental risk on human health.

Acknowledgements

This research is a part of the ongoing research program "Transdisciplinary approach to environmental risks", funded by the University of Nîmes, France. The authors would like to thank several vintners for helping to collect vineyard soil samples and grapevine components. The authors thank the editor and the anonymous reviewers for their constructive suggestions to improve the quality of the manuscript. Dr. H.M. Kluijver edited the final English version of the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.09.222.

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