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Lead Contamination in Portuguese Red Wines from the Douro Region: from the Vineyard to the Final Product

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To quantify lead contamination in wines and to try to identify major lead sources, two winemaking processes were followed during one annual cycle of wine production. Two vineyards from the Douro Portuguese region and two types of wine, one red table wine, which has been produced in a very modern winery, and one red fortified wine (similar to Port), which has been produced by a traditional vinification process, were selected for this study. Aerosols from the vineyards atmosphere, vineyard soil, vine leaves, grapes, and samples from the intermediary and final wine product were collected. Suitable pretreatments, namely, high-pressure microwave assisted digestion (soil, leaves, and grapes) and UV-irradiation (grape juices and samples from the different steps of the vinification processes), were used. The samples were analyzed in terms of lead total concentration and respective isotope ratios by using inductively coupled plasma mass spectrometry and atomic absorption spectrophotometry with electrothermal atomization. It was observed that the major sources of lead were in the vinification system, the more traditional one introducing more lead than the modern one. For the fortified wine, the lead concentration increased from 4.7 μg L^{-1}, in the grape juice, to 17.2 μg L^{-1}, in the final product, while for the table wine the increase was from 4.1 to 13.1 μg L^{-1}. Therefore, only about 1/4 (fortified wine) and 1/3 (table wine) of the lead total content of the final products came from soil and atmospheric deposition. Therefore, it is expected that marked reductions of the lead content in the wines would occur if the sources of lead were removed from the tubes and containers used in the vinification system, particularly by using welding alloys and small fittings free of lead. The lead levels in the vine leaves (global mean of 0.43 μg $g_{dry\ leave}^{-1}$) and grapes (global mean of 35 ng $g_{dry\ grape}^{-1}$) were similar in both vineyards.

KEYWORDS: Wine; lead; contamination; lead isotope ratios; vinification

INTRODUCTION

The regular absorption of small amounts of lead may originate serious effects on human health, particularly in individuals at risk. Lead present in wines is mainly associated with macromolecules, mainly tannin, polysaccharide, and protein fragments (*1*−*3*). In red wines, after digestion, only a fraction less than 20% of total lead seems to be present in assimilable forms (*4*, *5*). Even so, the International Office of Vine and Wine (OIV) has been reducing progressively the threshold limit value of lead total concentration in wines, which is actually 200 μg L^{-1}. Therefore, the control of the lead concentration in wine is required.

The lead present in the wine can result from two sources of contamination, one natural, soil related, and another resulting from human activity. The latter is related to pesticides, materials used to produce, transport, and store the wine and atmospheric precipitation of lead previously emitted by industry and transportation. To be able to reduce the level of lead in wine, it is important to know the relative relevance of the different sources of this element and to evaluate their contribution to the lead contamination in the final wine product. The few works found in the literature concerning this subject indicated that the major lead contamination came from anthropogenic sources. Up to leaded gasoline was forbidden in the 90's, atmospheric deposition was a main lead source in wines (*6*, *7*). At present, the contribution of transportation for the lead levels in the atmosphere is much smaller than in the past and results of the natural lead content of the combustibles used in the motors.

Both Kaufmann (*8*) and Rosman et al. (*9*) concluded that brass (a lead alloy that used to be widely utilized in traditional wine cellars) was the main lead contamination source in wines. The modernization of wineries led to the gradual replacement of brass by stainless steel that resulted in gradually lower lead levels in wine. Nevertheless, the wines produced presently still have significant levels of lead, and it is important to know all of the sources of this metal to remove or minimize them.

Lead is composed of four stable isotopes, three of which are of radiogenic origin: the radioactive decay of ^{238}U, ^{235}U, and ^{232}Th generates, respectively, ^{206}Pb, ^{207}Pb, and ^{208}Pb. The most

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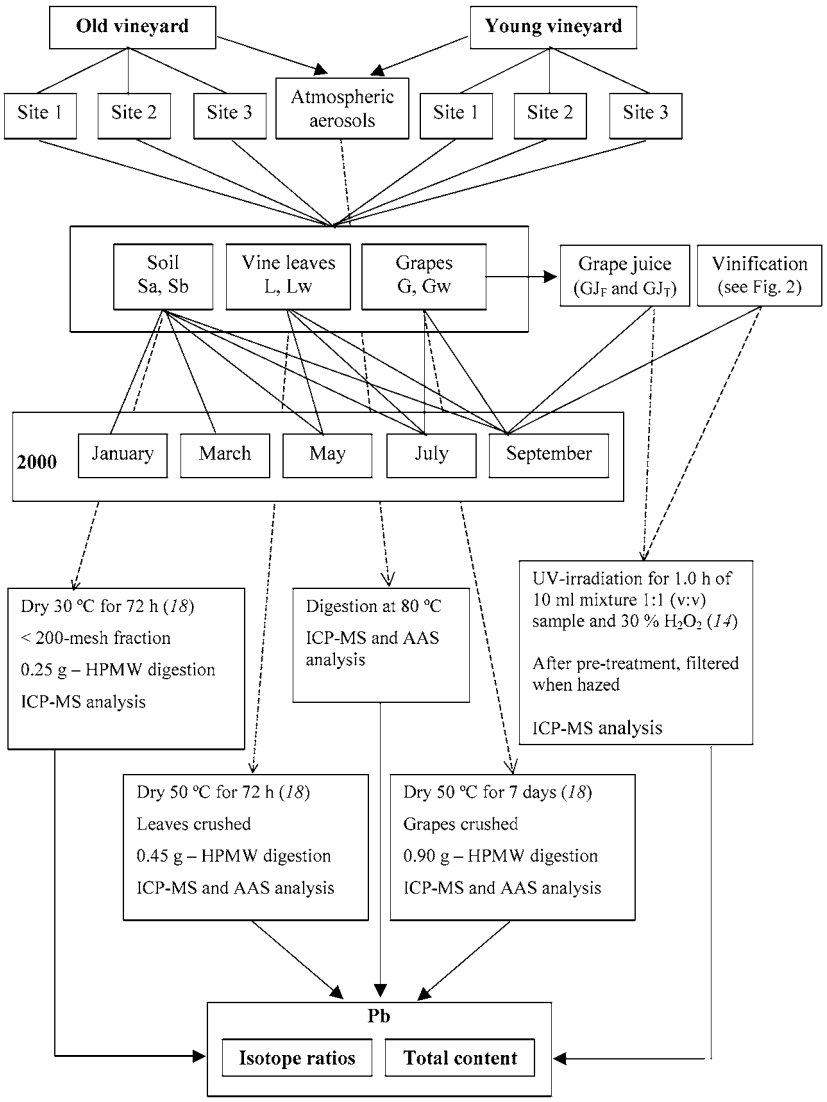

Figure 1. Sampling strategy and analytical procedures used in this work. S_a and S_b: soil samples collected at surface and 20 cm depth, respectively; L and G: nonwashed vine leaves and grapes, respectively; L_w and G_w: washed vine leaves and grapes, respectively; GJ_F and GJ_T: grape juice prepared in the laboratory by smashing grapes from the old and from the young vineyard, respectively.

stable isotope, ^{204}Pb, is nonradiogenic. The proportions of the lead isotopes vary with geological ages and consequently with the geographical origin of the element. Therefore, measurements of isotope ratios of lead may be explored to identify the sources of lead contamination.

The objective of this work was to identify possible sources of lead and their relative relevance in the lead content of young wines produced in a modern winery. For comparison, an old-fashioned wine production process was also carried out. For this purpose, the lead levels and the respective isotopic composition were determined in all the phases of the wine production procedure: from the vineyard soil and air to the final product ready to be sold to the consumers. Vine leaves, grapes, and samples collected throughout the vinification process were also monitored during an annual cycle of wine production (year 2000).

For isotope ratio measurements, inductively coupled plasma mass spectrometry (ICP-MS) was used. In the last years, this technique has been used for the determination of lead isotope ratios in table (*7*, *10*−*13*) and fortified (*14*, *15*) wines and in different environmental samples, including in studies were those ratios were used as tracers of pollution sources (e.g., refs *16* and *17*).

EXPERIMENTAL PROCEDURES

Materials and Reagents. Suprapure concentrated HNO_3 (65% m/m, $d = 1.40$ g mL^{-1}) and a solution of 30% H_2O_2, pro analysis, from Merck, were used without further purification. A stock NIST SRM-981 Common Pb Isotopic Standard solution (1000 mg L^{-1} of Pb) was prepared by dissolving a portion of the metal in 1% v/v HNO_3. For total Pb concentration (Pb_{total}) determinations, a pro analysis stock standard solution (1000 mg L^{-1}) from Merck was used. For ICP-MS internal standardization, Tl standard solutions were prepared from a pro analysis stock standard solution (1000 mg L^{-1}, from Alfa, for ICP-MS). All the other reagents were pro analysis grade or equivalent.

Standard solutions were prepared daily from the stocks, in polyethylene tubes, by weight, with deionized water (resistivity >14 MΩ cm) or diluted HNO_3 (see below). To avoid contamination, all material used for sampling and sample treatments was soaked in 20% v/v HNO_3 for at least 24 h, rinsed several times with deionized water, and dried in a Class 100 laminar flow hood. To collect the samples, no metallic instruments were used. The sample manipulation was carried out in a clean room with Class 100 filtered air.

Sampling. The sampling strategy is schematically described in Figure 1, which also includes resumed information on sample treatments. Two vineyards, one 60−70 years of age (old vineyard) and the other 10 years old raised in a forestal area (young vineyard), from the Douro Region (schistous soil) northeast of Portugal, were selected for this

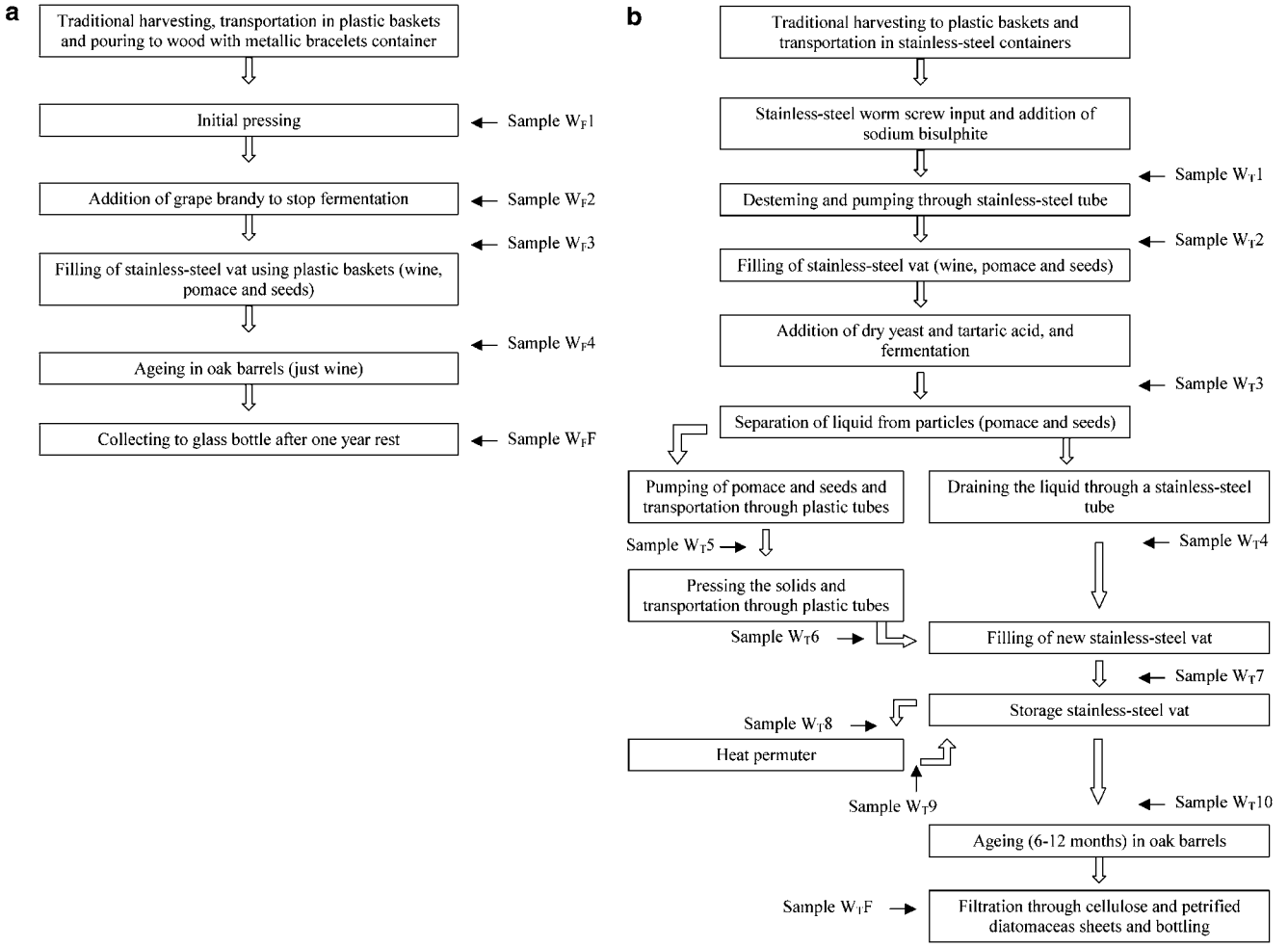

Figure 2. Vinification processes with indication of the points were wine samples were collected. (A) Red fortified wine produced from grapes of the old vineyard (W_F1 to W_F4 and W_FF); (B) red table wine produced from grapes of the young vineyard (W_T1 to W_T10 and W_TF).

study, which took place in the year 2000. In both vineyards, only treatments with copper sulfate solutions (which usually are not free of Pb contamination (*19*)) were carried out during the study. Nevertheless, in previous years, several treatment products (pesticides and fertilizers) had been used, particularly in the old vineyard. The vineyards are located in an agricultural area, mainly also vineyards, far from industrial activities, having a road with moderate traffic nearby.

The grapes, from the polyvarietal vines that have been grown in the old vineyard, were used to produce a red fortified wine similar to Port wine. The vinification process used for that purpose (summarized in **Figure 2**A) was performed manually, in an old-fashioned way, without automatic controls and involved a small number of steps, the wine being poured directly from one container to the next. At the end of the vinification process, the fortified wine had been aging in oak barrels for periods between two and 20 years depending on the desirable wine quality. For the present study, the sample called final product was collected after only one year of aging. However, the Pb content could still change after that time since the oak barrels where wine is aged contain metallic bracelets.

Only Touriga Nacional vines have been grown in the young vineyard, and their grapes were used to produce a monovarietal red table wine. The vinification process, summarized in **Figure 2B**, was automatically performed and controlled, involving much more steps than those of the fortified wine. Stainless steel tubes and containers were used in most steps. Plastic containers and tubes (polyethylene, high-density polyethylene, and flexible PVC) were also used for the harvest and to transfer must and wine in same steps. Aging of the wine took place in oak barrels. At the end, the wine was stored in glass bottles and analyzed within a month.

Samples were selected to follow the entire pathway of Pb, from the vineyard to the final wine product: atmospheric aerosols, vineyard soil, vine leaves, and grapes, as well as intermediaries and final wines collected throughout the entire vinification processes. Atmospheric aerosols from the vineyards were collected, in duplicate, whenever vine leaves were collected, in a point representative of all of the studied area. For this purpose, a low volume sampler was used (noncommercial, kindly offered by the Lawrence Berkeley Laboratory, Berkeley University, CA) provided with a 0.8 μm pore size filter (47 mm of diameter) of nitro-cellulose, from Millipore. Approximately 75 h of sampling with an air flux of $\sim$11 L min^{-1} was used. The vineyard soil (ca. 300 g per sample), vine leaves (ca. 100 g per sample), and grapes (ca. 200 g per sample) were collected using plastic shovels for soil and plastic gloves for leaves and grapes and were stored in individual plastic bags, which were immediately closed. For each vineyard, samples were collected in three different sites, selected to be representative of the entire vineyard. In each site, soil was collected at the surface (S_a) and at a 20 cm depth (S_b), and both leaves and grapes from each site were divided in two parts: one was washed with deionized water while the other remained as collected for analysis. Washing with water can remove at least the particles deposited on the surface that are not chemically bound to it (a 4% CH_3COOH washing solution could be more efficient in removing adsorbed contamination (*19*) but was not used in this work). Throughout the entire vinification processes, wine samples were collected in triplicate, into polyethylene tubes, at points where a possible Pb contamination could be expected (see **Figure 2**).

Sample Treatments. The atmospheric aerosol samples were attacked with concentrated HNO_3 using a previously optimized procedure (*20*). After digestion, the final solutions were kept at 4 °C and analyzed within 48 h. Before analysis, suitable dilutions with deionized water were carried out. Blank filters were treated as a sample and analyzed to subtract the background signal.

The samples of vineyard soil, leaves, and grapes were first dried in an oven up to constant weight (*18*). Soil was fractioned and homogenized by sieving through nylon nets of 2 mm and 200-mesh. Only the fraction <200-mesh was analyzed. Leaves and grapes were crushed with gloved hands and homogenized. From each sample, three portions of about 0.25 $g_{dry\ soil}$, 0.45 $g_{dry\ leaves}$, and 0.90 $g_{dry\ grapes}$ were treated for analysis. The portions were digested by high-pressure microwave assisted program (HPMW), which was carried out in closed PTFE vessels using a MLS-1200 Mega system, from Millestone, coupled to an exhaust EM-30 of the same brand. The digestion program consisted of three steps of 5 min each at 250, 400, and 500 W, respectively. For soil samples this program was run twice, whereas for the remaining samples it was run only once. For soil samples digestion concentrated HNO_3 was used, based on the literature (*21*, *22*). The efficiency of the procedure was tested with standard soil reference material: San Joaquin Soil 2709, from the National Institute of Standards and Technology (NIST). The optimized procedure is a strong acid digestion that dissolves almost all elements that could become environmentally available (*22*). Leaves and grapes were attacked with concentrated HNO_3 and 30% H_2O_2, using literature data as a starting point (*21*). After HPMW digestion, the final solutions were kept at room temperature until analysis, which took place within two days. Before analysis, suitable dilutions with a solution containing Rh (only for Pb_{total} determinations in soils) or with deionized water (remaining determinations) were carried out.

Grape juice was prepared in the laboratory. For this purpose, identical quantities of nonwashed grapes collected in each of the three sampling sites of each vineyard were mixed and smashed (with gloved hands) in plastic cups. The skins and seeds remained intact and were rejected. The obtained juice was transferred to polyethylene tubes. Aliquots of nonfiltered grape juice as well as of nonfiltered samples from the vinification processes were pretreated by UV-irradiation (*14*). Suitable dilutions with a solution containing HNO_3 and Tl (for Pb_{total} determinations) or with 0.5% HNO_3 solution (for Pb isotope ratios, IRs, determinations) were carried out afterward.

For each sample, three independent replicates (with the exception of the aerosol samples with only two) were prepared for analysis. Blank solutions treated as samples were regularly analyzed to subtract the background signal.

ICP-MS Measurements. The analytical measurements were carried out in a Perkin-Elmer SCIEX Elan 5000 ICP-MS (Perkin-Elmer, Norwalk, CT) apparatus equipped with a cross-flow nebulizer, nickel cones, and a peristaltic sample delivery pump. The operating conditions for ICP-MS measurements were optimized daily by using a solution with 10 $\mu g\ L^{-1}$ of Mg, Rh, and Pb and monitoring the isotopes ^{24}Mg, ^{103}Rh, and ^{208}Pb. Since doubly charged ions were not a concern and oxide ions were considered unlikely in the *m*/*z* 204−208 region, the operating conditions that maximized the ion intensity for mass 208 were selected. Operating conditions used: RF power of 1200 W; sample uptake rate of 0.800 L min^{-1}; plasma flow rate of 15.00 L min^{-1}; nebulizer flow rate between 1.000 and 1.050 L min^{-1}; and auxiliary flow rate of 0.800 L min^{-1}. The ions lens settings (in arbitrary units) were P = 52, S2 = 24, B = 70, and E1 = 15. For signal stabilization, a sample read delay of 1.5 min was required. Between solutions of samples or standards, the sampling system was rinsed with 2% HNO_3 for 1.5 min.

Pb Total Concentrations. The Pb_{total} in the vineyard soil samples was measured using a semiquantitative ICP-MS multi-element procedure optimized previously, which was based on that for multi-element determinations in wines (*23*). A sample of soil reference material (San Joaquin Soil 2709) was analyzed together with the soil samples every working day and several times a day, during a period of about two months. Daily instrumental variations between 1.6 and 4.4% and long-term instrumental variations around 10% were observed in Pb.

For the measurement of Pb_{total} in the wine samples, including those of grape juices and from the vinification steps, the data acquisition procedure was adapted from the instrument manual (*24*). The Pb isotopes (the ones with higher isotopic abundance) were measured using 10 sweeps per reading, a dwell time of 100 ms, and five replicates per measurement, in peak hopping mode, at normal resolution. Since changes in the isotopic abundance between samples could occur, the obtained concentration in each sample replicate was the mean of the one obtained for each Pb isotope. External calibration was used, and the appropriate interpolation was carried out. In the final samples and standard solutions, 0.5% HNO_3 and 30 $\mu g\ L^{-1}$ Tl were always present. Tl was used for internal standardization to eliminate the matrix discrepancies and to compensate for any drift occurring during the analysis. The accuracy of the results was tested through an intercomparison with those obtained, in parallel, for two selected wines by the laboratory of the Instituto do Vinho do Porto in Portugal.

Table 1. Comparison of External and Internal Mass Bias Correction for Two Samples of Soil (S) and of Leaves (L)

	$^{207}Pb/^{206}Pb$[a]		$^{208}Pb/^{206}Pb$[a]		$^{204}Pb/^{206}Pb$[a]	
sample	external corrn	internal corrn	external corrn	internal corrn	external corrn	internal corrn
S_1	0.849 (5)	0.851 (4)	2.134 (11)	2.129 (8)	0.0544 (9)	0.0538 (3)
S_2	0.843 (5)	0.851 (3)	2.145 (10)	2.151 (9)	0.0540 (9)	0.0532 (3)
L_1	0.856 (3)	0.859 (4)	2.111 (12)	2.097 (10)	0.0549 (3)	0.0546 (3)
L_2	0.860 (5)	0.853 (3)	2.106 (15)	2.087 (10)	0.0549 (2)	0.0547 (4)

[a] Mean and standard deviation (in brackets affecting last digit, calculated according to errors propagation).

Pb Isotope Ratios. The data acquisition procedure for the measurement of the three Pb IRs in all samples, $^{204}Pb/^{206}Pb$, $^{207}Pb/^{206}Pb$, and $^{208}Pb/^{206}Pb$ (determined individually), was optimized previously for wine samples (*14*, *15*). The Pb isotopes were measured using 1500 sweeps per reading, a dwell time of 10 ms, and three replicates per measurement, in peak hopping mode, at normal resolution. The isotope ^{204}Pb was measured twice (by choosing the time factor 2 in the parameter file of the ICP-MS software) as long as the other Pb isotope signals and a mathematical correction of ^{204}Hg interference with this isotope was systematically carried out (*14*, *15*).

Mass Bias Correction for IRs Measurements. For the soil and leaves, mass bias corrections with both Pb isotopic standard (HNO_3 and Pb concentrations similar to that pre-estimated for the samples) and Tl IR (constant natural IR $^{205}Tl/^{203}Tl = 2.3871$) were carried out in parallel for two of the first collected samples, and the results were compared (see **Table 1**). No significant differences were obtained for all of the Pb IRs, similar results being expected for grapes. For wine samples, corrections of the Pb IRs values for mass bias with both the Pb isotopic standard and the Tl IR had been carried out in a previous work (*15*). No significant differences had been observed either between the results obtained using both types of corrections for the ratios of $^{207}Pb/^{206}Pb$ and $^{208}Pb/^{206}Pb$, but for the ratio of $^{204}Pb/^{206}Pb$, the correction with Tl IR was the only one suitable. Therefore, the ratio of $^{204}Pb/^{206}Pb$ in the grape juices and in all of the samples from the different steps of the vinification processes was corrected with Tl IR (measured simultaneously with the Pb IR in the sample). The remaining Pb IRs in these samples and all the Pb IRs in the other type of samples were corrected with the Pb isotopic standard. To underscore a possible shift with time, every workday the standard was analyzed first and for every two or three samples (for IR $^{204}Pb/^{206}Pb$ the standard was measured only every four to six samples because it was more time-consuming, and for this IR the repeatability was higher than for the other two IRs). The standard deviation associated to each measurement value was calculated according to propagation of errors (resulting from the determination of the Pb IR both in the sample and in the isotopic standard or from the Tl IR in the sample).

In a first stage, to test the repeatability of the results obtained for the soil samples (three replicates per sample), and since Pb_{total} did not change significantly among the different replicates (see below), the mean and variance of the Pb IRs of three independent replicates were calculated and compared with the mean and variance obtained for a single replicate, which was analyzed three times. The variance obtained for the Pb IRs in three independent replicates of each soil sample were of the same order of magnitude as that obtained for a single replicate (results are illustrated in **Table 2**). Similar results had been observed previously for wine samples (*14*) and indicated that the ICP-MS determinations were the decisive factor for the precision of the Pb IRs.

Table 2. Precision of the Pb IRs Observed in Two Different Soil Samples

		analysis of three aliquots of a single replicate	analysis of three independent replicates	$\sigma^{2\,a}$
		$^{207}Pb/^{206}Pb$		
S_1	mean	0.853	0.854	
	σ^2	3.1×10^{-5}	4.8×10^{-5}	1.7×10^{-5}
S_2	mean	0.853	0.850	
	σ^2	2.7×10^{-5}	2.2×10^{-5}	-5.6×10^{-6}
		$^{208}Pb/^{206}Pb$		
S_1	mean	2.123	2.120	
	σ^2	3.8×10^{-4}	3.4×10^{-4}	-4.1×10^{-5}
S_2	mean	2.115	2.118	
	σ^2	1.2×10^{-4}	1.2×10^{-4}	-1.2×10^{-5}
		$^{204}Pb/^{206}Pb$		
S_1	mean	0.0541	0.0541	
	σ^2	7.7×10^{-8}	8.1×10^{-8}	3.7×10^{-9}
S_2	mean	0.0538	0.0540	
	σ^2	2.3×10^{-7}	1.9×10^{-7}	-4.2×10^{-8}

[a] Difference between the total variance (analysis of three independent replicates) and the determined variance (analysis of three aliquots of a single replicate).

Table 3. ETAAS Furnace Program Used for the Determination of Pb in Atmospheric Aerosols, Leaves, and Grape Samples

temperature (°C)	ramp time (s)	hold time (s)	argon flow (mL min^{-1})
110	1	20	250
130	5	30	250
350	10	20	250
1500	0	5	0
2400	1	2	250

Therefore, for subsequent studies only one portion of each homogenized soil sample was processed for the determination of the respective Pb IRs.

ETAAS Analysis. The measurements by ETAAS were carried out in a Perkin-Elmer 4100 ZL apparatus with Zeeman background correction and coupled to an autosampler, model AS-70 of the same mark. The optimized furnace program (see **Table 3**) was based on the ones described in the instruments manual (*25*) and on former experiments. Regular analysis of Pb in blood (PbS) and atmospheric aerosols (MET) has been carried out in our laboratory integrated in intercalibration programs PICC (Programa Interlaboratorios de Control Calidad): PbS (from Gabinete de Seguridad e Higiene en le Trabajo, Zaragoza, Spain), and MET (from Centro Nacional de Condiciones de Trabajo, Barcelona, Spain).

For aerosol samples, external calibration with aqueous standard solutions was used, and an appropriate interpolation was carried out. For the vine leaves and grapes analysis, since no reference materials were available, the standard addition method was used.

Statistical Calculations. With the purpose of testing if among the analyzed samples significant differences occurred between the values of the Pb IRs (that is, whether the difference between two sample means was too great to be explained by the random error), statistical tests, using the one-tailed F-test for one-way ANOVA (*26*), were applied. For comparison of Pb_{total} among the different samples, one-way ANOVA tests were carried out using the software package SPSS 10.0 for Windows.

RESULTS AND DISCUSSION

Total Pb Concentrations. *Atmospheric Aerosols.* The Pb contents in atmospheric aerosols in the vineyards area were relatively low, around 18 ng m_{air}^{-3} in May (during a dry and sunny weather period) and around 7.5 ng m_{air}^{-3} in July and September (during more wet weather with rainy periods), being much lower than the threshold limit value fixed in the Portuguese legislation, 2.0 $\mu g\ m_{air}^{-3}$. The observed variations in Pb concentrations were probably related to the weather conditions since rain facilitates the deposition of the aerosols thus decreasing the concentration of contaminants in the air.

Vineyard Soil. The levels of Pb_{total} observed in the vineyard soil (monthly values) are shown in **Figure 3**. The Pb contents were similar at surface and at 20 cm depth, but they were slightly higher in the old vineyard, between 13.1 ± 0.7 and 22 ± 1 $\mu g\ g_{dry\ soil}^{-1}$ (standard deviations, $n = 3$) with a global mean of 17 $\mu g\ g_{dry\ soil}^{-1}$, than in the young vineyard, between 9 ± 1 and 17.1 ± 0.7 $\mu g\ g_{dry\ soil}^{-1}$ with a global mean of 13 $\mu g\ g_{dry\ soil}^{-1}$. Pesticides contaminated with Pb probably enriched the old vineyard soil in this metal.

In a few months, the levels of Pb_{total} in one of the three sampling sites of each vineyard were significantly higher (and higher than the equipment experimental long-term variation, 10%, and daily variation, 1.6−4.4%) than those observed in the other two sites. However, such differences probably only reflected the heterogeneity of the metal distribution in the soil instead of a specific anthropogenic contamination. Systematic significant differences in the Pb_{total} were not found either among the three sampling sites or during the year.

Leaves and Grapes. **Figure 4** shows that the Pb_{total} content in the vine leaves varied between 0.15 ± 0.05 and 0.62 ± 0.02 $\mu g\ g_{dry\ leaves}^{-1}$, with a global mean of 0.42 $\mu g\ g_{dry\ leaves}^{-1}$, in the old vineyard and between 0.22 ± 0.04 and 0.7 ± 0.2 $\mu g\ g_{dry\ leaves}^{-1}$, with a global mean of 0.43 $\mu g\ g_{dry\ leaves}^{-1}$ in the young vineyard. Therefore, the Pb_{total} levels were similar in both vineyards, despite Pb_{total} in soil being higher in the old one.

In both vineyards, in most cases, the monthly values of Pb_{total} were lower in the washed leaves than in those nonwashed, although the differences were statistically significant only for the samples collected in July and September in the young vineyard. In many cases, the Pb_{total} levels were higher in September than in May or July. This fact indicates that the Pb concentration in the leaves slightly increased with age.

The results obtained for grapes are shown in **Figure 5**. The Pb_{total} contents, between 26 ± 14 and 52 ± 21 ng $g_{dry\ grape}^{-1}$, with a global mean of 40 ng $g_{dry\ grape}^{-1}$ in the old vineyard and between 17 ± 8 and 57 ± 20 ng $g_{dry\ grape}^{-1}$, with a global mean of 30 ng $g_{dry\ grape}^{-1}$ in the young vineyard, were similar in both vineyards. These levels were about 1 order of magnitude lower than those found in the leaves. It is known that the different organs of a plant show different abilities to accumulate metals, seeds and fruits accumulating less metal than leaves and roots in most species (*27*).

In contrast with the leaves, the Pb_{total} in the grapes did not increase from July to September. However, these data may be only apparent, resulting in an incomplete drying of the mature grapes collected in September (despite that they had been dried in an oven up to a constant weight) because of the presence of a very high sugar content.

Statistically significant differences in the Pb_{total} levels in grapes collected in different sites of both vineyards as well as between washed and nonwashed grapes were not found. The reproducibility of all the data regarding grapes was poor (relative standard deviations, RSDs, of 50% in some cases) probably because of the either low Pb concentrations or to variation in the degree of hydration of the grapes.

Grape Juices and Samples from the Vinification Processes. The levels of Pb_{total} determined in the grape juices prepared at the laboratory (GJ_F and GJ_T for the fortified and table wine,

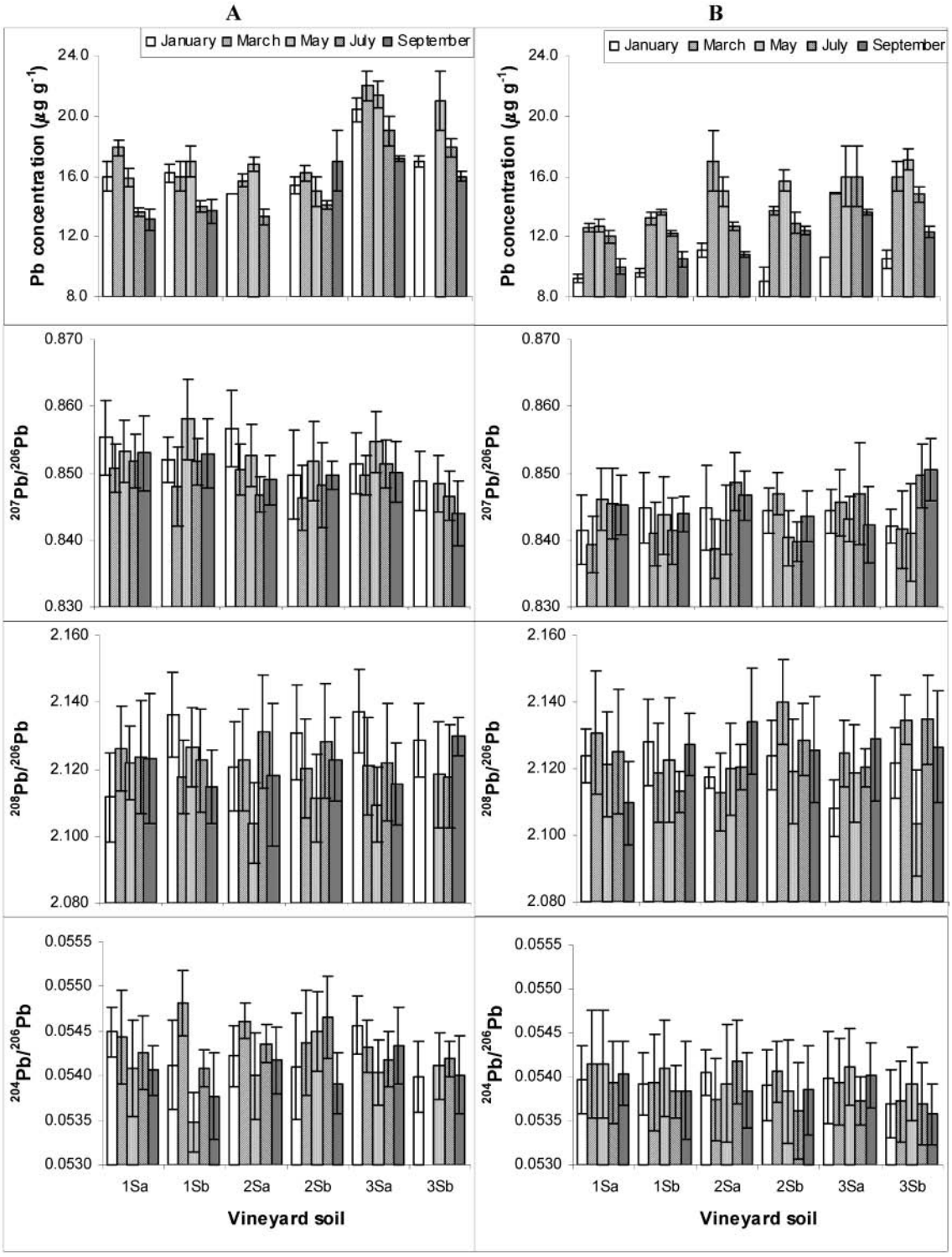

Figure 3. Levels of Pb_{total} (in μg per g of dry soil) and respective IRs obtained in the vineyard soil samples collected at surface (S_a) and at 20 cm depth (S_b), in the three selected sites (1S to 3S), in the old (A) and young (B) vineyards, in five different months. The sample $3S_b$ from the old vineyard and from March was lost. Mean and standard deviation ($n = 3$) are presented.

respectively) and in the different samples collected during and at the end of the vinification processes (see **Figure 2**) are presented in **Figure 6**. For the fortified wine (from the old vineyard), the concentration of Pb increased about 265%, from 4.7 ± 0.3 μg L^{-1} in the GJ_F to 17.2 ± 0.3 μg L^{-1} in the final product (W_FF). For the table wine (from the young vineyard) the Pb concentration increased about 220%, from 4.1 ± 0.5 μg L^{-1} in the GJ_T to 13.1 ± 0.1 μg L^{-1} in the final product (W_TF). These marked increases in the Pb levels during the vinification procedures indicated that at least in the studied area (Portuguese Douro region) environmental contamination was a minor source of the Pb found in the wines. Even the very modern vinification system, used to produce the red table wine, introduced significant amounts of Pb in the final product, although lower than those introduced by the old-fashioned process used to produce the fortified wine. It must be noted that the Pb concentration in the studied wines was much lower than the threshold limit value established by the OIV (200 μg L^{-1}).

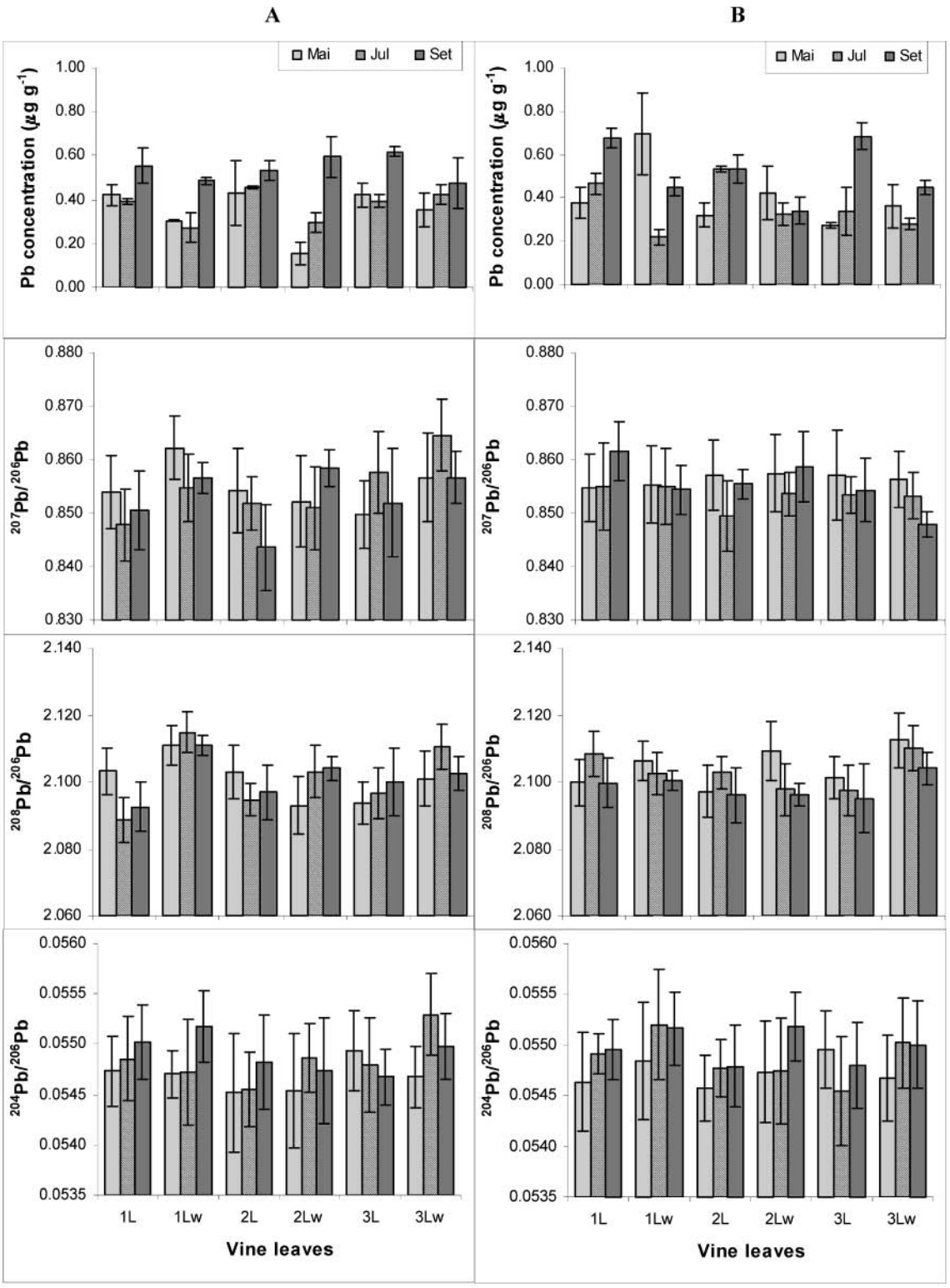

Figure 4. Levels of Pb_{total} (in μg per g of dry vine leave) and respective IRs obtained in the vine leave samples washed (L_w) and nonwashed (L) collected in the three selected sites (1L to 3L), in the old (A) and young (B) vineyards, in three different months. Mean and standard deviation ($n = 3$) are presented.

A more detailed analysis of the data of **Figure 6** shows that the increase in Pb_{total} throughout the vinification processes was very regular, with only a few exceptions. Main sources of Pb were probably the alloys used to weld pieces of the different containers and tubes used in the vinification system (see **Figure 2**) as well as same fittings, like traps. In addition to contamination, liberation of Pb from grape skins and seeds may also have enriched the samples with Pb during the first stages of vinification (pressing and fermentation). Teissedre et al. (*19*) has reported significant differences in the levels of Pb in the different parts of the grape berries, having in the seeds the highest content of Pb and in the pulp the lowest one.

In the case of the fortified wine, a slight but significant decrease in the Pb concentration occurred between samples W_F1 and W_F2, which was due to the addition of grape brandy (practically free of Pb) to stop the fermentation, resulting in a

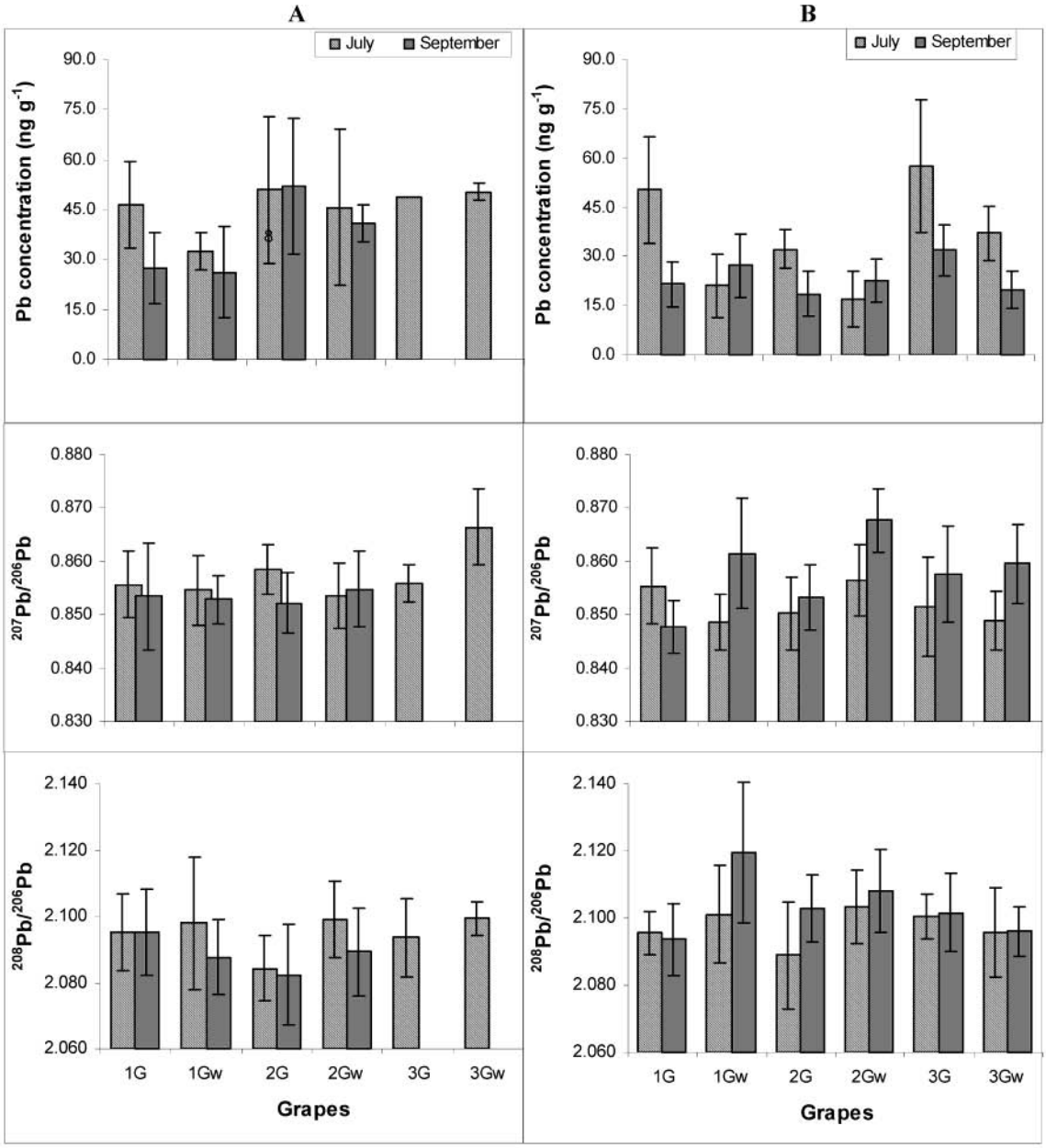

Figure 5. Levels of Pb_{total} (in ng per g of dry grape) and respective IRs obtained in the grape samples washed (G_w) and nonwashed (G) collected in the three selected sites (1G to 3G), in the old (A) and young (B) vineyards, in two different months. The samples 3G and $3G_w$ from the old vineyard and from September were lost. Mean and standard deviation ($n = 3$) are presented.

20% (v/v) dilution of the product. In the following steps of the vinification, the Pb level increased again. Solubilization of Pb complexes of organic ligands because of the addition of brandy, releasing Pb from skins and seeds that hung with pomace during and after fermentation, and contamination from the metallic bracelets of the container were expected to be the major Pb sources. The Pb level attained a maximum in sample W_F4 when it rested in a stainless steel vat, which possibly had some sources of Pb, like welding strings. Afterward, the particles (pomace and seeds) were removed, and the liquid was transferred to oak barrels where it aged for one year, after which the last sample was collected (W_FF) for analysis. The Pb_{total} in W_FF was markedly lower than in W_F4 probably because a large fraction of Pb was chemically bound with some colloidal polymeric organic compounds and was removed with the solid phase or coprecipitated with it. The precipitation or coprecipitation with suspended particles during fermentation and/or aging has been reported in the literature for several elements including Pb in other wines (*28*, *29*).

During the vinification of the table wine, a progressive increase in Pb_{total} was observed that, besides release of the metal from skins and seeds in the first vinification steps (W_T1 to W_T3), was also probably related to the presence of Pb sources in the stainless steel of the tubes and vats. From W_T2 to W_T3 a significant and marked increase of Pb content occurred. However, from W_T3 to W_T4 there was a decrease of Pb, indicating that some metal was removed with the particles (pomace and seeds) since the liquid extracted from the pressing of the solids (W_T5) was slightly richer in Pb than the previous separated liquid phase (W_T4).

Therefore, the available data on Pb_{total} clearly demonstrated that the major contribution for the Pb levels in the studied wines came from the vinification processes. As concerns the Pb fraction that was already present in the grapes, the Pb IRs may

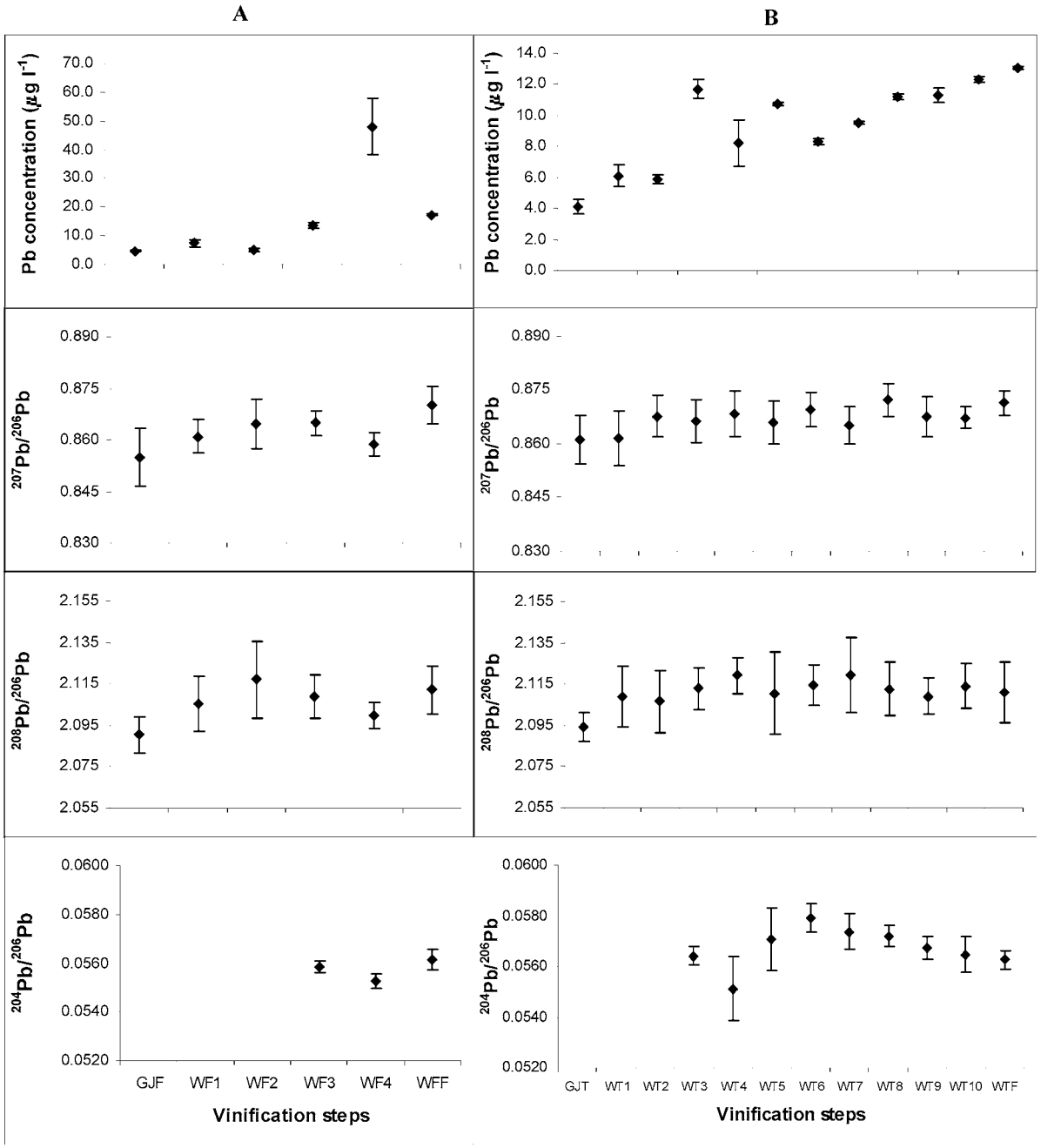

Figure 6. Levels of Pb_{total} (in $\mu g\ L^{-1}$) and respective IRs obtained for the grape juices (GJ_F and GJ_T for the fortified and table wine, respectively), for all the samples collected throughout the vinification processes (W_F1 to W_F4 for the fortified and W_T1 to W_T10 for the table wine) and for the final products (W_FF and W_TF for the fortified and table wine, respectively). (A) Red fortified wine produced with grapes from the old vineyard; (B) red table wine produced with grapes from the young vineyard. The ratio $^{204}Pb/^{206}Pb$ could not be determined in the samples GJ, W1, and W2 of both wines. Mean and standard deviation ($n = 3$) are presented.

give some information on the relative relevance of soil and other atmospheric particles as Pb sources.

Lead Isotope Ratios. *Atmospheric aerosols.* The values of the ratios $^{207}Pb/^{206}Pb$, $^{208}Pb/^{206}Pb$, and $^{204}Pb/^{206}Pb$ were measured in the aerosols collected from the atmosphere of the vineyards in May, July, and September. The Pb isotopic composition was identical in the three months (results not shown), suggesting that the atmospheric Pb sources were similar in those sampling dates.

Vineyard Soil. The values of the three Pb IRs observed in the vineyard soil are included in **Figure 3**. For $^{204}Pb/^{206}Pb$ the RSDs ($n = 3$) associated to the means, between 0.35 and 1.2%, were worse than those obtained for the remainders IRs ($\leq$0.85% for $^{207}Pb/^{206}Pb$ and $\leq$1.0% for $^{208}Pb/^{206}Pb$), presumably entirely because of the poor counting statistic on the ^{204}Pb isotope since this is the least abundant isotope. Similar results were observed before in wine samples (*14*, *15*) and also for the other types of samples analyzed in this study.

Results of statistical tests indicated that, for each vineyard, no significant variations of the Pb IRs occurred either throughout the months or between the two soil layers: surface (S_a) and 20 cm depth (S_b). Therefore, eventual anthropogenic contamination, resulting for instance of the vine treatment, could not be identified. These results are consistent with the constant level of Pb_{total} observed in the vineyards soil.

Vine Leaves. For each vineyard, significant changes did not occur in the Pb IRs measured either in the leaves throughout the vineyard or during the period of study (see **Figure 4**). These

Table 4. General Average Values of Pb IRs, with Respective Standard Deviation (Value in Brackets Affecting Last Digit) Calculated for the Different Types of Samples

	Old vineyard			Young vineyard		
	$^{207}Pb/^{206}Pb$	$^{208}Pb/^{206}Pb$	$^{204}Pb/^{206}Pb$	$^{207}Pb/^{206}Pb$	$^{208}Pb/^{206}Pb$	$^{204}Pb/^{206}Pb$
			Soil			
S_a[a]	0.852 (3)	2.121 (8)	0.0543 (2)	0.844 (3)	2.121 (7)	0.0540 (1)
S_b[a]	0.850 (3)	2.123 (7)	0.0541 (1)	0.844 (3)	2.125 (9)	0.0538 (2)
			Leaves			
L[a]	0.851 (1)	2.097 (4)	0.0548 (1)	0.855 (2)	2.100 (4)	0.0548 (1)
L_w[a]	0.857 (2)	2.106 (3)	0.0549 (1)	0.855 (2)	2.104 (4)	0.0550 (1)
			Grapes			
G[a]	0.855 (3)	2.090 (1)	ND[b]	0.851 (1)	2.096 (4)	ND[b]
G_w[a]	0.856 (1)	2.095 (5)	ND[b]	0.857 (2)	2.106 (4)	ND[b]
aerosol[c]	0.862 (3)	2.102 (6)	0.0554 (4)			

[a] S_a and S_b: soil samples collected at surface and 20 cm depth, respectively; L and G: nonwashed vine leaves and grapes, respectively; L_w and G_w: washed vine leaves and grapes, respectively. [b] ND: not determined. [c] Collected in a point representative of both vineyards.

results corroborated the previous conclusions based on the levels of Pb_{total}.

Regarding washed and nonwashed leaves, also no statistically significant differences between them were found. Therefore, eventual contamination by atmospheric deposition from sources other than soil could not be clearly identified through the Pb isotopic signature.

Grapes. The very low content of Pb present in grapes of both vineyards prevented the determination of the IR $^{204}Pb/^{206}Pb$. Therefore, only $^{207}Pb/^{206}Pb$ and $^{208}Pb/^{206}Pb$ were measured (**Figure 5**). Similar to that observed for leaves, no significant change occurred for the two Pb IRs in the grapes throughout each vineyard, the period of study and between washed and nonwashed grapes.

Grape Juices and Samples from the Vinification Processes. The values of $^{207}Pb/^{206}Pb$ and $^{208}Pb/^{206}Pb$ were determined in all the samples collected throughout the vinification processes, where eventual Pb contamination could be introduced (see **Figure 2**). The ratio $^{204}Pb/^{206}Pb$ was only determined in the latest points (from W3 to WF for both types of wines) (**Figure 6**), owing to the too low Pb_{total} of the remaining sampling points.

Despite that the Pb_{total} increased during these vinification processes, the variations of the Pb IRs values were within the errors associated to the analytical measurements, preventing the identification of the Pb sources. This fact resulted in a combination of relatively low level of Pb contamination with the presence of different Pb sources probably with distinct Pb isotopic signatures, which masked each other.

Comparison of Pb IRs in the Samples of Different Origins. When the values of the different IRs of an element measured in various samples are plotted one against the other, a simple source of the element will form a cluster on the graph, while linear arrays tend to appear if several disparate sources are mixed (*30*). With the purpose of trying to extract some valuable information about the Pb sources, the general mean values of each Pb IR in the different types of samples from each vineyard (see **Table 4**) and from the respective vinification process (see **Figure 6**) were compared by plotting (*y* vs *x*): $^{208}Pb/^{206}Pb$ versus $^{207}Pb/^{206}Pb$, $^{204}Pb/^{206}Pb$ versus $^{207}Pb/^{206}Pb$, and $^{204}Pb/^{206}Pb$ versus $^{208}Pb/^{206}Pb$. The results are shown in **Figure 7** (panel A: fortified and panel B: table wines).

For the fortified wine, it was observed that the old vineyard soil and the atmospheric aerosols displayed Pb of different isotopic compositions. Vine leaves presented not very different Pb isotopic composition from that of grapes, being this composition a mixture of those present in soil, in aerosols, and probably in a third component (not analyzed in this work) since it got out of the linear array formed by soil and aerosol samples. Such a third component may be related to pesticides and/or fertilizers used in previous years in this vineyard, which have been accumulating in the soil surrounding the vines. Regarding the samples collected in the different steps of the vinification, some changes in the Pb IRs occurred during the process. For instance, GJ_F and grapes had similar IRs, which differed from those of W_F2 and W_F3, while W_F1 presented a Pb isotopic composition between GJ_F and W_F2 or W_F3. These results are compatible with the fact that during the first steps of the vinification the must with pomace and seeds had been in a wood container with metallic bracelets, while GJ_F, produced at the laboratory, had no contact with metallic devices. Therefore, some Pb from metallic alloys with Pb isotopic composition different from that of GJ_F contaminated the samples. This result is in agreement with the increase in Pb_{total} observed in those samples. The sample W_F4, which was collected after the wine (together with pomace and seeds) had rested in a stainless steel vat, presented different Pb IRs than the previous samples. This suggests that the container had also some sources of Pb different from the previous ones, which is corroborated by the high increase observed in Pb_{total} at this step. The Pb IRs of the final product, W_FF, were also different from all the previous ones, suggesting the presence of a different Pb source of contamination during the one-year rest in an oak barrel.

Regarding the table wine/young vineyard, it was observed that soil and aerosols presented markedly different Pb isotopic compositions. Vine leaves presented a Pb isotopic composition similar to that of the grapes, which seemed to be mostly a mixture of those present in soil and in the atmospheric aerosols. The influence of a third component, probably related to pesticides and/or fertilizers used in previous years and accumulated in the soil surrounding the vines, was much less evident in this case than in the old vineyard, as both leaves and grapes fitted more or less in the linear array formed by soil and aerosol samples. This is compatible with the fact that the young vineyard had been submitted to much less chemical treatments in the past than the old vineyard. Some differences were observed between grapes and GJ_T. However, these differences resulted probably because of instrumental errors (related to the low Pb content of the samples) since GJ_T, prepared in the laboratory, had no contact with metallic devices, and therefore, isotopic composition identical to that of the grapes would be expected as it was observed for the fortified wine. As concerns the vinification process, the sample W_T1 presented Pb isotopic composition different from GJ_T and from the subsequent samples. Considering the significant increase in Pb_{total} that was observed between GJ and W_T1, it could be concluded that there was already some Pb contamination in this step, probably from Pb sources present in the stainless steel containers used to transport the grapes after the harvest. The Pb isotopic signature observed in that sample (W_T1) resulted probably because of a nonhomogenized mixture of different isotopic compositions. This is compatible with the fact that different isotopic compositions were observed in W_T1 and W_T2, despite having a Pb_{total} similar and the must in these steps having only a small contact with stainless steel containers. The samples collected during the remaining steps of vinification, including W_T2, formed a cluster. This suggested that only one type of source of Pb contamination was present in the stainless steel tubes and vats used in most of the steps of the modern vinification system.

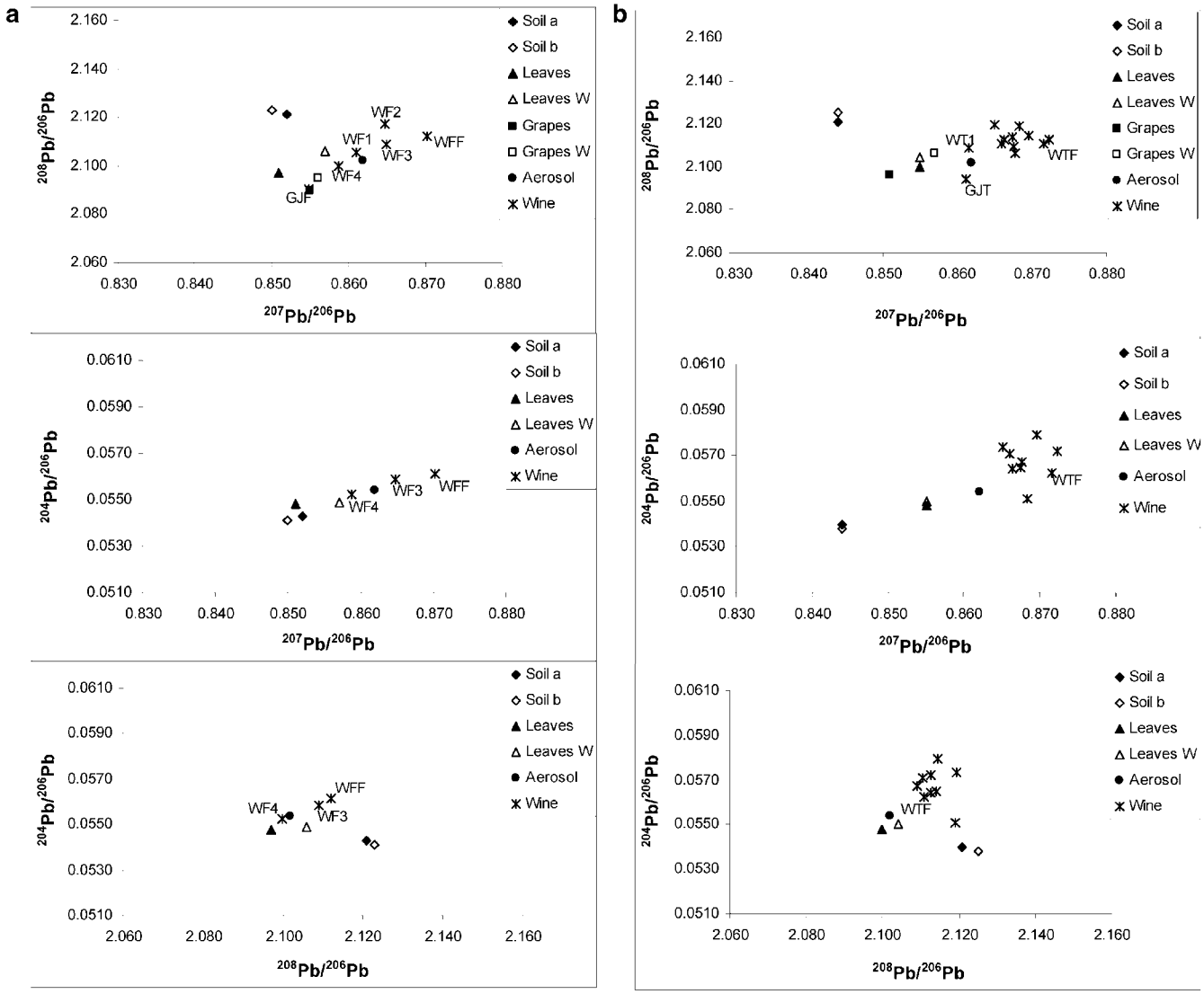

Figure 7. Plots of $^{208}Pb/^{206}Pb$ vs $^{207}Pb/^{206}Pb$, $^{204}Pb/^{206}Pb$ vs $^{207}Pb/^{206}Pb$, and $^{204}Pb/^{206}Pb$ vs $^{208}Pb/^{206}Pb$ for comparison between the mean values of the Pb IRs of the different samples: surface (♦) and 20 cm depth (◇) vineyard soil; nonwashed (▲) and washed (△) vine leaves; nonwashed (■) and washed (□) grapes; atmospheric aerosols (●); grape juices (GJ_F and GJ_T for the fortified and table wine, respectively), all samples collected throughout the vinification processes (W_F1 to W_F4 for the fortified and W_T1 to W_T10 for the table wine) and final products (W_FF and W_TF for the fortified and table wine, respectively) (*). (A) Red fortified wine produced from grapes of the old vineyard; (B) red table wine produced from grapes of the young vineyard.

This is in agreement with the fact that the Pb concentration increased proportionally to the vinification steps, as discussed previously.

CONCLUSIONS

Both studied wines contained relatively low levels of Pb. However, the Pb levels in the fortified wine, which was produced by a traditional vinification process, were higher, 17.2 $\mu g\ L^{-1}$, than in the table wine, 13.1 $\mu g\ L^{-1}$, which was produced according to modern technology. In both cases, the Pb levels were much lower than the threshold limit value established by the International Office of Vine and Wine (200 $\mu g\ L^{-1}$).

The major sources of the lead contamination were found in the vinification processes. Soil and direct atmospheric deposition only contributed about 1/4 (fortified wine) or 1/3 (table wine) to the Pb content of the wines. Some of the Pb present in the musts seems to be removed by precipitation or coprecipitation with particles during the vinification processes. No significant differences were observed among the Pb IRs determined in the different types of samples because of the relatively high standard deviations associated with the measurements. However, when the values of the different Pb IRs were plotted one against the other, different sources of contamination became visible, indicating that the major sources of Pb were in the different containers and devices used throughout the vinification process. Therefore, those plots showed to be valuable tools for differentiating Pb isotopic composition, giving, in this case, interesting information about Pb sources of contamination in wines.

This study indicates that a drastic reduction of the Pb level in the wine would be possible by a very strict control of the Pb sources in the devices used in the vinification system, particularly in the alloys used in welding processes and in small fittings, like taps.

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