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Risk Assessment on Irrigation of *Vitis vinifera* L. *cv Malbec* with Hg Contaminated Waters

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Supporting Information

ABSTRACT: Concerns regard watering crops with Hg contaminated waters have arisen worldwide recently. In these sense Hg uptake by *Vitis vinifera* L. *cv. Malbec* was evaluated under greenhouse conditions by the administration of Hg^{2+} for 4 days through irrigation water (short-term administration). Vines uptake Hg translocating it from roots through stems to leaves. Roots accumulated the higher Hg concentration. Hg in stems and leaves was accumulated mostly as organic Hg, bind to different moieties. Size exclusion chromatography (SEC) and ion pair chromatography (IPC) were employed to reach insights into these ligands. Hg is distributed mainly in high molecular weight fractions of 669 kDa in vine plants. In stems and leaves, Hg−S associations were found in 669 and 66 kDa fractions. Hg−S association at 66 kDa suggests a possible protein or peptide binding affecting vines normal physiology. Since Hg contamination through organomercurials is more harmful than Hg^{2+} itself, methyl mercury, dimethyl mercury, and phenyl mercury, more toxic Hg species were evaluated with negative results.

1. INTRODUCTION

Mercury has an average crustal abundance on Earth of approximately 0.05−0.10 mg kg^{-1}, the majority of which occurs at the mineral cinnabar.[1] Natural sources and transport mechanisms include volcanic emissions, wind borne dust, geysers, thermal fluids, and sea-spray.[1,2] Hg has been extensively used in the production of electrical goods, pulp and paper products, paints, dental applications, and pesticide formulations. About half of the anthropogenic input to the environment, including irrigation channels, has come from the manufacturing of caustic soda and chlorine by the electrolysis of brine.[3] Volatility of some Hg species has turned this metal into a global pollutant which has been measured in the deep ocean, the atmosphere, Antarctica and the Arctic.[1]

Even in the absence of direct exposure, toxic elements represent a hazard to human population, because the food chain connects the elements of soil and air with humans. Uptake and accumulation by plants represents the main entry pathway for potentially health-threatening toxic metals into human and animal food. The exposure to Hg both directly and through the food chain is of significant concern and has resulted on more than one occasion in remedial response activation in regions of the U.S. and in other parts of the world.[4,5]

It has been documented that plants absorb elements which have no known biological function and are even known to be toxic at low concentrations. Among these are As, Cd, Cr, Hg, and Pb. However, even micronutrients become toxic for plants when absorbed above certain threshold values. Metal−ion contamination is a serious type of pollution in the environment. For plants, it can induce development problems such as growth decrease, reduced biomass production, and other morphological and biochemical alterations.[6,7] Plants uptake essential and nonessential elements from soils in response to concentration gradients induced by selective uptake of ions by roots, or by diffusion of elements in soil.[8] Bioavailability refers to the ability of an element to be transferred from the soil to a living organism.[9] Evaluating bioavailability should provide information regarding risks of contaminant transfer and accumulation into the food chain.[9−12]

Viticulture represents an important agricultural practice in many countries.[13] Metals in wine define its origin, and more important, its quality. They contribute to the formation of opacity and to the color, aroma and taste of wines. The final content is therefore the result of a number of different variables, such as the chemical and physical characteristics of the soil in which vines have grown.[14] The irrigation water quality is another important variable defining the transportation of metals and other elements necessary for vine growth from soil toward the plant.[15] Irrigation with contaminated waters can seriously increase the probability of toxic metals uptake into vine plants.

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Irrigation channelss can be contaminated from different sources like industrial effluents,[16] wastewaters,[17] and geological processes.[18]

The metal uptake capacity of *Vitis vinifera* has been reported elsewhere.[19−21] Elevated heavy metal concentrations were found in xylem saps of vines showing a relatively high mobility within the plants.[20] Chopin et al.[19] studies showed differences between elements uptake resulted from vegetation uptake strategies and soil partitioning. In addition, it has been demonstrated that Hg content in vines cultivated in Hg contaminated soils is elevated compared with vines grown in soils with a normal Hg concentration.[21]

Despite the fact that many works have studied vines metal uptake from contaminated soils, little is known about the possibility of crops irrigation with Hg contaminated waters. The aim of this work is the assessment of Hg uptake and distribution by *Vitis vinifera* L. *cv. Malbec* through short-term Hg supplementation to vines cultivated in green house conditions. Studies of Hg biotransformation into different plant organs will be performed through the analysis of total Hg, organic and inorganic fractions, and distribution according to molecular weight. Hg analysis was also expanded to the search of more toxic Hg substances like $MeHg^+$, Me_2Hg and $PhHg^+$. Information about how vines, a major global crop, metabolize Hg after exposure has not been fully explored or understood.

2. EXPERIMENTAL SECTION

2.1. Procedures. *2.1.1. Plants Cultivation and Supplementation.* Plants were obtained from Estación Experimental Agropecuaria Mendoza, Instituto Nacional de Tecnología Agropecuaria (INTA). The experiment was carried out at Facultad de Ciencias Agrarias, Universidad Nacional de Cuyo, Mendoza, Argentina (33°0′S, 68°52′W) at an altitude of 940 m. One-year-old plants of a selected clone of *Vitis vinifera* L. *cv. Malbec* were planted in 1.0 L plastic pots filled with 450 g of grape compost. Grape compost consists of three parts of pomace, two parts of loam, and two parts of perlite (pH: 7.2; conductivity: 18.3 mΩ cm^{-1}; organic matter 9%). They were grown in a green house at temperatures ranging from 23 and 27 °C (night and day). In order to reproduce a situation where vines are irrigated with Hg contaminated water, the short-term supplementation procedure reported by Afton et al.[22] was adapted with modifications. Plants were split into four groups of three plants each. Three groups were supplemented with Hg^{2+} (as $HgCl_2$) at 25 mL d^{-1} for 4 days as depicted: 10 mg L^{-1}; 50 mg L^{-1}; 100 mg L^{-1}, and the rest one was employed as control and no Hg was added. Plants were allowed to mature for one additional week before sampling. The health of each plant was visually indifferent to the supplementation given. During the process of sampling, plants were separated into roots, stems, and leaves. They were immersed in an ultrasonic bath for a complete soil removal, washed with ultrapure water, and lyophilized. Fine roots were chosen for Hg uptake analysis as fine roots accumulated higher trace element concentrations; they would provide more accurate data on vegetation response to trace element presence in the environment and should, thus, be selected for bioavailability studies.[19] Finally, the different plant organs were stored at −5 °C to prevent any further enzymatic activity leading to interspecies conversion, therefore changing the native distribution. Soil samples were collected after Hg supplementation in order to determine the root bioavailable Hg concentration and the total Hg content. After collection, soil samples were lyophilized and stored at −5 °C to prevent any further enzymatic activity.

2.1.2. Extraction Procedures. A mild extraction procedure of Hg from vine tissues was adapted from Meng et al.[23] to reach extraction of free Hg and Hg bond to different ligands in the plant. 0.3−0.5 g of vine plants organs samples were digested using a mortar followed a KOH-methanol/solvent extraction technique. In this process, vegetal organ samples were first digested with a KOH (5%, w v^{-1}) − CH_3OH (50%, v v^{-1}) solution and heated at 50 °C in a water bath for 3 h. After completion, extracts were centrifuged at 3500 rpm for five minutes. The supernatant was acidified with HPO_3.

Soil samples were treated following the procedure reported by Cattani et al.[24] for the extraction of Hg bond to different ligands. One g of soil was mixed with 9 mL HCl (7.6%, w v^{-1}) and 1 mL mercaptoethanol (10%, v v^{-1}) in a polycarbonate bottle. The mixture was placed in an ultrasonic bath for 45 min, with addition of ice to avoid an excessive warming of the bath water, and then centrifuged for 5 min at 3000 rpm. Following centrifugation the supernatant pH was adjust to 7.0 with ammonia (10%, w v^{-1}). Finally, before analysis, both extracts, from vine and soil samples, were filtered through a 0.45 μm membrane. Bioavailable Hg was determined by weighting 1 g of soil then shaken with 10 mL of 0.05 mol L^{-1} EDTA solution, pH 5.0 for 2 h on a rotary shaker. After that the mixture was centrifuged (4000 rpm) and filtered prior analysis.

For the assessment of Hg distribution by size exclusion chromatography (SEC), an extraction stage involving liquid nitrogen and a mortar followed by the addition of 2 mL of a 2% (w v^{-1}) SDS - 30 mM Tris solution was performed. Extraction was completed after a 2 h ultrasonication bath and centrifugation at 5000 rpm at 4 °C. Supernatant was collected and filtered through 0.22 μm filter prior injection for analysis.[25]

2.1.3. Microwave Assisted Digestion. The microwave digestion for total Hg determination in vine organs samples was performed as follows: 0.5 g were weighed and placed in individual microwave graduated polystyrene tubes. The aliquots were treated with 7 mL of HNO_3 65% (v v^{-1}) and 1 mL of H_2O_2 (3:1, v v^{-1}). Dissolution was carried out at a ramp temperature of 10 min up to 200 °C and hold for 10 more minutes. The employed microwave power was up to 1000 W.

2.1.4. Total Hg and Organic/Inorganic Fraction Determination. In order to reach organic Hg (Hg_{org}), Hg bond to different ligands; and inorganic Hg (Hg_{inorg}), Hg^{2+} or free, determination, a 100 μL injection valve and rotary pumps were employed to propel the sample to an UV−CV−AFS system (Figure S1 of the Supporting Information (SI)). For determination of Hg_{inorg} (as Hg^{2+}), HCl 30% (v v^{-1}), and $SnCl_2$ 10% (m v^{-1}) in HCl 30% (v v^{-1}) were introduced to the system. For Hg_{org} fraction determination, $K_2S_2O_8$ 1% (m v^{-1}) in 30% (v v^{-1}) HCl, $NaBH_4$ 0.5% (m v^{-1}) in 0.5% NaOH were introduced into the system under UV irradiation. Hg_{org} decomposition by this procedure has been assessed before.[26] Under these conditions it has been stated that Hg_{org} in different concentrations values reach a decomposition range of 95.3−99.7%.[26]

Total Hg determinations were performed in the microwave assisted digests of root, leave, and stem samples. The employed technique was ICP MS. Two mL of the sample digests were introduced into the system. Determinations were carried out directly in the digests.

2.1.5. Ion Pair and Size Exclusion Chromatography. Determination of $MeHg^+$, Me_2Hg, and $PhHg^+$ was performed

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by ion pair chromatography (IPC) coupled to UV−CV−AFS. Separation conditions were adapted from Cattani et al.[25] with modifications. Operating conditions of the LC pump are summarized in Table S1 (SI). 2-Mercaptoethanol added in the mobile phase can be used as anion-pair reagent, forming complexes with Hg^{2+}, $MeHg^{+}$, and $PhHg^{+}$ in order to reach the separation of these compounds. Chemically, Hg^{2+}, $MeHg^{+}$, and $PhHg^{+}$ have extremely strong affinities for sulfhydryl-containing ligands.[24]

The separation was achieved employing a gradient mode elution. First, a mobile phase (A) of 100% (v v^{-1}) buffer phosphate pH 7.0; 2-mercaptoethanol 0.1% (v v^{-1}) was introduced to reach the separation of Hg^{2+} and $MeHg^{+}$. Once Hg^{2+} was eluted (551 s) the mobile phase was changed to 65% (v v^{-1}) buffer phosphate pH 7.0; 2-mercaptoethanol 0.1% (v v^{-1}) and 35% (v v^{-1}) methanol (B) to reach the elution and separation of $PhHg^{+}$ and Me_2Hg. B mobile phase was increased at a rate of 2.5% min^{-1} in order to change the strength of the gradient. AFS technique shows a great advantage in comparison with ICP MS because it can tolerate the introduction of solutions with a higher content of organic solvents such as methanol, like in this separation [35% (v v^{-1})], keeping comparable detection limits. Figure 4a shows the optimized separation.

SEC was performed coupling the chromatographer to ICP MS. Buffer ammonium acetate 50 mM was employed being adequate for coupling with ICP MS, since its volatility do not generate deposits on ICP cones. Bovine serum albumin (66 kDa), Alcohol dehydrogenase (150 kDa), β-amilase (200 kDa), Thyroglobulin (669 kDa), and Apoferrtin (443 kDa) were employed for calibration.

2.2. Statistical Analysis. All samples were collected and analyzed in duplicate and the duplicate tests were statistically similar as paired-samples t test ($p = 0.05$). The average results were used to represent the data. Microsoft Excel was used to test one-way analysis of variance (ANOVA) at 95% confidence to investigate the effect of Hg concentration into irrigation water on Hg uptake capacity by vines.

The least significant difference (LSD) was calculated as follows:

$$\mathrm{LSD} = \sqrt{\sigma_0^2} \times \sqrt{\frac{2}{n}} \times t_{\mathrm{h}(n-1)} \quad (1)$$

3. RESULTS AND DISCUSSION

3.1. Total Hg Determinations. Total Hg determinations were performed in three aliquots of the whole plants after a total microwave assisted digestion. Determinations were made for the three supplementation groups and the control group. Results can be observed in Figure 1. Vines uptake Hg and transport it from roots to leaves. The maximum Hg concentration value was found for the group supplemented with 100 mg L^{-1} corresponding to 86.43 ± 15.98 μg g^{-1}, followed by group supplemented with 50 and 10 mg L^{-1} corresponding to 66.08 ± 11.31 and 29.06 ± 3.56 μg g^{-1}, respectively (for all determinations results are expressed as the mean ± standard deviation of three plants). This fact is in agreement with previous studies where it was described a higher Hg uptake when vines where grown in Hg contaminated soils,[21] however the elevated Hg concentrations are overwhelming considering the low time of exposure, only 4 days. This can be explained considering the high mobile nature of the Hg source, the irrigation water. Elevated Hg concentration values were found in roots, stems, and leaves compared with those grown in soil with lower Hg levels. The control group showed concentrations of 0.04 ± 0.01, 0.02 ± 0.01, and 0.02 ± 0.01 μg g^{-1} for roots, stems, and leaves, respectively.

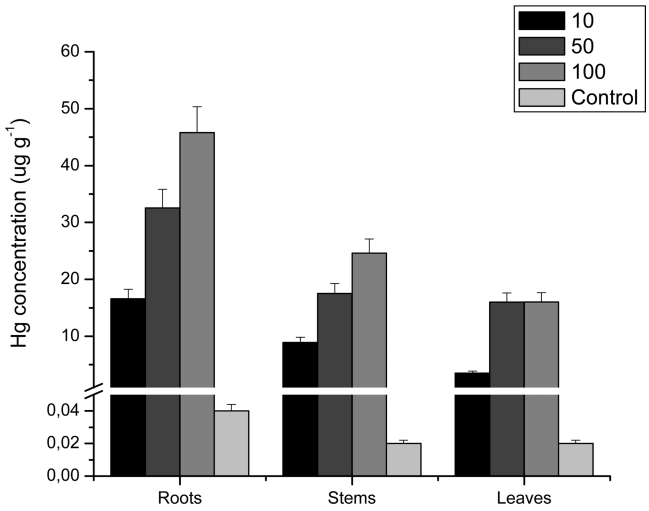

Figure 1. Total Hg concentration into the different vine compartments evaluated for 10, 50, and 100 mg L^{-1} of Hg^{2+} supplementation. Results are expressed as the mean ± standard deviation of three plants.

From the analysis of the aerial and nonaerial organs of the plant it can be observed that Hg concentrations in roots were of 16.61 ± 2.34, 32.57 ± 4.3, and 45.77 ± 6.2 μg g^{-1}, for groups supplemented with 10, 50, and 100 mg L^{-1}, respectively. No significant difference was observed between groups supplemented with 10 and 50; and 50 and 100 mg L^{-1} (LSD = 17.58 μg g^{-1}). A similar trend is advised in leaves, corresponding to 3.52 ± 1.1, 16.0 ± 3.9, and 16.05 ± 4.1 μg g^{-1} of Hg concentration, respectively, with no significant difference among groups supplemented with 10 and 50; and 50 and 100 mg L^{-1} (LSD = 12.93 μg g^{-1}). Stems showed concentrations corresponding to 8.93 ± 2.1, 17.51 ± 4.9, and 24.61 ± 6.7 μg g^{-1}, respectively, with no significant difference among groups supplemented with 10 and 50; and 50 and 100 mg L^{-1} (LSD = 19.21). Statistical results obtained from ANOVA (Table 1) show significant variation (CI = 95%) of Hg concentration in roots, stems, and leaves regard different Hg concentrations in irrigation water.

Higher Hg concentrations in roots can be explained considering that the defense mechanism of plants against heavy metals is to hold metals in the root to avoid damage to aerial organs.[12] In addition it has been suggested that it is possible that plants are able to contribute to the release of Hg to the air by taking up Hg from the soil, translocating it to the leaves and releasing it via the stomata.[27]

3.2. Determinations of Hg_{org} and Hg_{inorg}. Extraction of Hg_{org} and Hg_{inorg} fractions employing the described procedure in section 2.1.2 was assessed with recoveries between 44 and 60% compared with complete digestion. The fractions were evaluated employing $SnCl_2$ as reducing reagent to determine only Hg_{inorg} (as Hg^{2+}), and by the introduction of $NaBH_4$, $K_2S_2O_8$, and UV radiation into the FI system, the decomposition of both fractions, Hg_{org} and Hg_{inorg} was achieved. Hg_{org} was calculated by difference.

Hg distribution into Hg_{inorg} and Hg_{org} fractions can be observed in Figure 2 and Table 2 for each study group and

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Table 1. One way ANOVA for the Effect of Hg Concentration in Irrigation Water on Hg Uptake by *Vitis vinifera*[a]

effect	roots			stems			leaves		
	dF	F	*p*	dF	F	*p*	dF	F	*p*
Hg concentration in irrigation water	6	30.91[b]	0.0006	6	7.56[b]	0.022	6	14.12[b]	0.005

[a]F_{critic} = 5.14 (p = 0.05). [b]Significant at 95% confidence level.

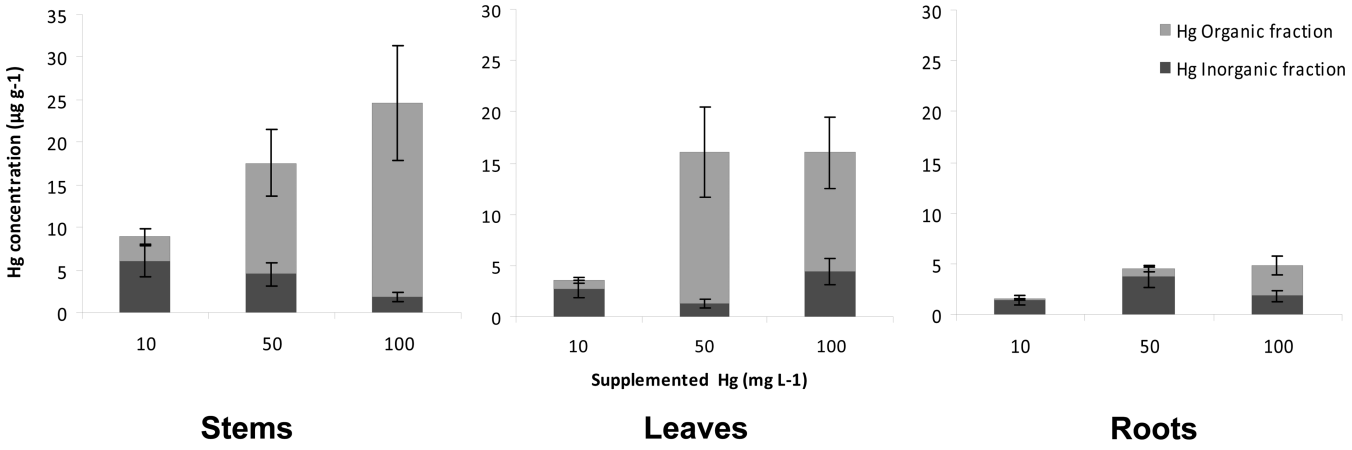

Figure 2. Hg_{org} and Hg_{inorg} concentration into the different vine compartments evaluated for 10, 50, and 100 mg L^{-1} of Hg^{2+} supplementation. Results are expressed as the mean ± standard deviation of three plants.

Table 2. Hg_{org} and Hg_{inorg} Distribution in the Different Vine Organs

	Hg_{org} and Hg_{inorg} distribution ($\mu g\ g^{-1}$)[a]							
	roots		stems		leaves		total	
supplemented Hg^{2+} (mg L^{-1})	Hg_{org}	Hg_{inorg}	Hg_{org}	Hg_{inorg}	Hg_{org}	Hg_{inorg}	Hg_{org}	Hg_{inorg}
10	0.06 ± 0.02	1.57 ± 0.3	2.84 ± 0.7	5.42 ± 0.9	0.82 ± 0.3	2.4 ± 0.15	3.67 ± 0.6	9.28 ± 1.9
50	0.71 ± 0.1	3.61 ± 0.9	13.05 ± 3.0	4.75 ± 1.3	14.73 ± 3.6	1.86 ± 0.12	28.49 ± 6.3	11.7 ± 2.5
100	3.03 ± 1.2	2.01 ± 0.2	22.76 ± 4.2	1.93 ± 0.2	11.64 ± 3.5	5.17 ± 0.6	37.42 ± 9.2	10.89 ± 2.4

[a]Results are expressed as the mean ± standard deviation of 3 plants.

plant organs. Different distribution of Hg_{org} was observed for the studied groups. The highest Hg_{org} concentration corresponds to the group supplemented with 100 mg L^{-1} and 50 mg L^{-1} of Hg^{2+}, with 37.42 ± 9.2 $\mu g\ g^{-1}$ and 28.49 ± 6.3, respectively, (no significant difference, LSD = 25.05), followed by the group supplemented with 10 mg L^{-1}, 3.67 ± 0.6 $\mu g\ g^{-1}$. As it can be observed, the presence of Hg_{org} in vine plants is not proportional to the quantities of supplemented Hg into irrigation waters. Hg_{org} distribution within the different organs of vines showed that stems possess the highest Hg_{org} concentration with 22.76 ± 4.2 $\mu g\ g^{-1}$ corresponding to the group supplemented with 100 mg L^{-1}. The highest Hg_{org} found in stems suggests that one response of vines to Hg stress is the active translocation of this metal from roots to leaves through stems. Toxic metals taken up into the root reaches the xylem for upward transport.[28] Roots on the other hand showed the lowest Hg_{org} content with 0.06 ± 0.02 $\mu g\ g^{-1}$ for the group supplemented with 10 mg L^{-1}. These Hg_{org} concentration values are correlated with Hg translocation and storage process from nonaerial to aerial organs of vines due to the lowest Hg_{org} found in roots compared with higher Hg_{org} levels found in stems and leaves.

Hg_{inorg} and Hg_{org} concentration into the different organs of vine plants, nonaerial and aerial, can be observed in Table 2 and Figure 2. Vine plants supplemented with 10 mg L^{-1} of Hg^{2+} showed a lower Hg_{org} concentrations in the nonaerial and aerial organs of the plant compared with other studied groups. On the other hand, when Hg stress is heavier, the presence of Hg_{org} is elevated in stems and leaves, between 72.47 and 92.48% (considering the sum of Hg_{inorg} and Hg_{org} equal to 100%). These observations are explained by Cobbet and Goldsbrough[29] who stablished that Hg_{org} complexes are able to induce the production of Hg ligands in a positive feedback system. The two best-characterized heavy metal-binding ligands in plant cells are the phytochelatins (PCs) and metallothioneins (MTs).[29] In vivo studies have shown that PC synthesis can be induced by a range of metal ions in both intact plants and plant cell cultures.[30] PCs and MTs are peptides and proteins rich in sulfur residues able to form thiolate bonds with Hg,[31] being responsible of sequestering Hg to avoid toxic effects.

3.3. Size Exclusion Chromatography Analysis. As described previously, Hg is present in stems and leaves of vines supplemented with 100 mg Hg L^{-1}, mainly as Hg_{org}. SEC analysis was applied to determine Hg distribution within this fraction. S was determined simultaneously in order to investigate the presence of Hg–S complexes inferring the presence of peptides and proteins[32] like PCs or MTs for instance. The employed SEC column separates in a wide range from 10 to 700 kDa. Since the extraction was performed with a TRIS-SDS solution, water-soluble Hg compounds such as proteins, polysaccharides, amino acids, polypeptides, and Hg protein complexes were extracted with TRIS. Water-insoluble Hg protein complexes were extracted with SDS (an anionic tensoactive solution).[33] Hg extraction efficiency compared with

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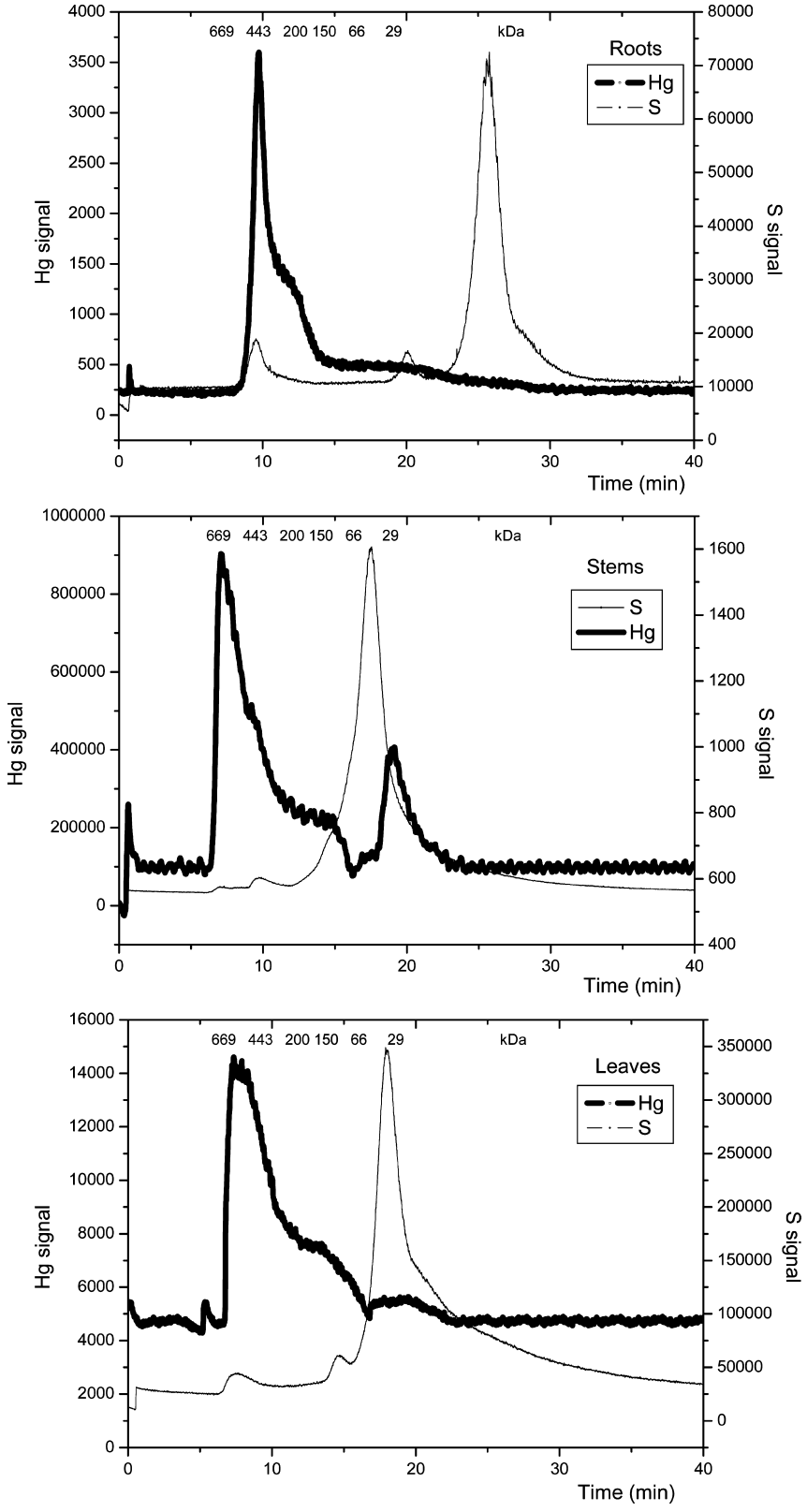

Figure 3. SEC–ICP MS chromatograms of plant compartments supplemented with 100 mg L^{-1}. Molecular weight markers can be observed in the upper side of graphics.

total Hg determinations was 0.47, 0.12, and 2.8% in roots, stems, and leaves, respectively. Formation of Hg–S complexes was confirmed by the injection of a 2-mercaptoethanol-Hg solution.

Figure 3 shows SEC chromatograms of roots, leaves, and stems extracts supplemented with 100 mg L^{-1} of Hg^{2+}. The studied plant organs show that in the Hg_{org} fraction, this metal is bond mainly to high molecular weight fractions from 669 to

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443 kDa. Since the molecular weight is too high, protein binding in this range is disregarded. Fractions containing S also appear at this high molecular weight range, but at lower concentrations compared with medium and low molecular weight fractions, ~66 kDa and lower. Hg−S complexes are also present at these levels suggesting a possible Hg binding to proteins or peptides in stems. In roots and leaves Hg is present only in high molecular weight fractions. Hg is not associated to S in medium and low molecular weight fractions despite S presence.

Hg concentrations in the extracts were of 0.018 ± 0.003, 0.041 ± 0.001, and 0.38 ± 0.07 μg g^{-1} in roots, stems, and leaves, respectively of plants supplemented with 100 mg L^{-1} of Hg^{2+}. This tendency is coincident with Hg_{org} concentration, were higher Hg_{org} is observed in stems and leaves.

3.4. Ion Pair Chromatography Analysis. As stated in the previous section, high levels of Hg_{org} into stems and leaves in vines for the studied groups were found. In an attempt to elucidate the composition of Hg_{org}, the presence of Hg species was evaluated through IPC with UV−CV−AFS system as Hg detection. The different Hg species analyzed were Hg^{2+}, $MeHg^+$, Me_2Hg, and $PhHg^+$, and they can be observed in Figure 4. Analysis of these species becomes relevant considering their high toxicity and bioavailability level, even higher than Hg^{2+}. The only Hg specie found was Hg^{2+}. As an example the only chromatogram showed is the corresponding to leaves extract analysis. Hg species analysis confirms that vine plants do not methylate Hg^{2+}. Meng et al.[23] observations established that $MeHg^+$, Me_2Hg, or $PhHg^+$ are exogenous to plants, they do not synthesize them. Since the only supplemented Hg specie was Hg^{2+}, unless $MeHg^+$, Me_2Hg or $PhHg^+$ were added into the irrigation water, or were present in soil due to other mechanisms (like methylation by microorganisms for instance), they will not be present in vine plants organs. This was confirmed by the analysis of a possible Hg methylation in soil after Hg supplementation. The only Hg specie found in soil available to root after the extraction procedure at the harvesting time was Hg^{2+}. Concentrations of Hg^{2+} found were ranging from 0.21 to 0.49 μg g^{-1}, similar to those found by Maserati and Ferrara[34] in soils near a chlor−alkali complex, showing a clear contaminated soil after Hg supplementation through irrigation water, reaching in a greenhouse, field conditions. Despite these results, the uptake of $MeHg^+$ or other organic forms of Hg by vine plants could not be confirmed by this study. Beyond this statement, it is clear that Hg methylation did not occur in soil or plants at least one week after supplementation.

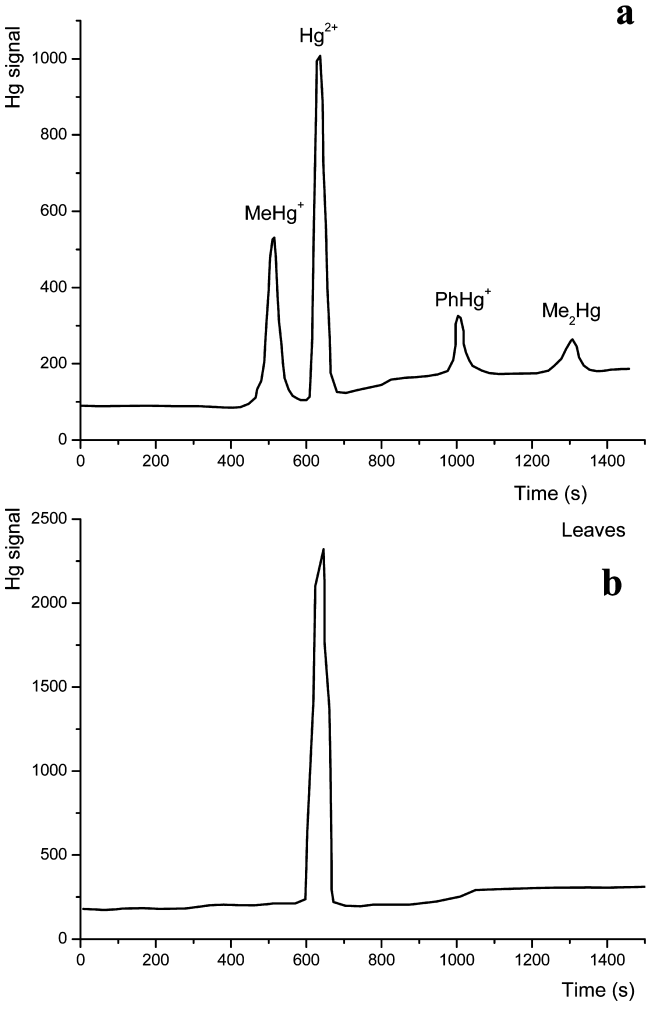

Figure 4. IPC−UV−CV−AFS chromatograms. (a) Standards injection of 50 μg L^{-1} of $MeHg^+$ (446 s), Hg^{2+} (551 s), $PhHg^+$ (875 s), Me_2Hg (1138 s). b) Sample injection (leaves), Hg^{2+} (551 s), supplemented with 100 mg L^{-1} Hg.

3.5. Evaluation of Uptake Parameters. In order to evaluate the Hg uptake capacity of vine plants and Hg transfer up to aerial parts of the plant, in Table 3 are depicted the bioaccumulation factor (BAF) (vegetation/soil) and transfer (aerial parts/fine roots) coefficients in *Vitis vinifera* L. *cv. Malbec*.

Table 3. Accumulation (Vegetation/Soil) Ratios and Transfer (Aerial Parts/Fine Roots) Coefficients in Vine Plants

supplemented Hg^{2+} (mg L^{-1})	bioavailable Hg (μg g^{-1})	bioaccumulation Factor (BAF)[ba]	transfer coefficient[ca]
10	0.011 ± 0.001	1509.9 ± 270.7	0.07
50	0.043 ± 0.005	757.4 ± 128.9	0.10
100	0.08 ± 0.006	572.2 ± 94.6	0.08

[a]Results are expressed as the mean ± standard deviation of three plants. [b]BAF = Hg concentration in root/bioavailable Hg in soil). [c]Transfer coefficient = Hg in aerial compartments/Hg in nonaerial compartments.

BAF provide comparison between different types of vegetation and soils (element concentration in vegetation root/available Hg concentration in soil).[9] In addition, since there is a lack of data for *Vitis vinifera* L., therefore, BAF could provide comparison between different types of vegetation and soils and provide a better understanding of the relationship between available Hg concentration in soil and Hg concentration in vegetation.[19] As shown in Table 3, BAF values correspond to 1509.9 ± 270.7, 757.4 ± 128.9, and 572.2 ± 94.6 for groups supplemented with 10, 50, and 100 mg L^{-1}, respectively. BAFs are similar, despite being different species, to those values obtained by Moreno-Jiménez et al.[36] for Hg uptake by *Rumex induratus* and *Marrubium vulgare*, employing the same BAF formula and Hg administration. BAFs for Hg uptake have not been reported for *Vitis vinifera* L. before.

Since trace elements accumulated in roots could be translocated to other plant organs[12] the transfer coefficient of the different vine groups are shown in Table 3. The determined

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values correspond to 0.74 ± 0.17, 1.02 ± 0.22, and 0.89 ± 0.17 for groups supplemented 10, 50, and 100 ug L^{-1} of Hg, respectively. The similarity between these transfer coefficients can be explained considering that the studied plants are clones. These transfer coefficients turn *Vitis vinifera* into an indicator for Hg contamination in soils according to Baker classification[35] (accumulators, TC > 1.5; indicators, TC from 0.5 to 1.5, and excluders, TC < 0.1)

4. ENVIRONMENTAL RELEVANCE

Irrigation waters can be contaminated from different sources like industrial effluents, wastewaters, and geological processes. Since Hg can be released from natural sources and from different industries like manufacturing of caustic soda and chlorine, this metal can reach aqueducts or irrigation channels. It was shown that vines uptake Hg proportionally to a short-term supplementation. Once absorbed, Hg distributes in roots, stems, and leaves, mostly as organic fraction of 669−443 and ∼66 kDa in stems. Within this last fraction Hg is associated to sulfur, indicating protein or peptide binding. Once Hg is absorbed it was demonstrated that it is not metabolized into more hazardous species like $MeHg^+$, Me_2Hg, or $PhHg^+$, under these experimental conditions, with a Hg short-term exposure.

■ ASSOCIATED CONTENT

Supporting Information

Additional information on reagents and instrumentation, inductively coupled plasma mass spectrometry (ICP MS), atomic fluorescence spectrometry (AFS) and high performance liquid chromatography (HPLC) is available. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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