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Characterization of Hg-phytochelatins complexes in vines (*Vitis vinifera cv Malbec*) as defense mechanism against metal stress

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Abstract An approach to understand vines (*Vitis vinifera*) defense mechanism against heavy metal stress by isolation and determination of Hg-phytochelatins (PCs) complexes was performed. PCs are important molecules involved in the control of metal concentration in plants. PCs complex toxic metals through −SH groups and stores them inside cells vacuole avoiding any toxic effect of free metals in the cytosol. The Hg-PCs identification was achieved by determination of Hg and S as hetero-tagged atoms. A method involving two-dimensional chromatographic analysis coupled to atomic spectrometry and confirmation by tandem mass spectrometry is proposed. An approach involving size exclusion chromatography coupled to inductively coupled plasma mass spectrometry on roots, stems, and leaves extracts describing Hg distribution according to molecular weight and sulfur associations is proposed for the first time. Medium–low molecular weight Hg–S associations of 29–100 kDa were found, suggesting PCs presence. A second approach employing reversed-phase chromatography coupled to atomic fluorescence spectrometry analysis allowed the determination of Hg-PCs complexes within the mentioned fractions. Chromatograms showed Hg-PC_2, Hg-PC_3 and Hg-PC_4 presence only in roots. Hg-PCs presence in roots was confirmed by ESI–MS/MS analysis.

Keywords *Vitis vinifera* · Mercury · Phytochelatins · Metal stress

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Introduction

Mercury (Hg) is one of the most toxic elements and has adverse impact on the human health and the environment. Mercury pollution is a ubiquitous problem resulting both from natural events and anthropogenic activities (Finkelman et al. 2002; Finkelman 2004; Wilcox et al. 2012; Dai et al. 2012). Natural sources and transport mechanisms include volcanic emissions, wind borne dust, geysers, thermal fluids, and sea-spray (Fergusson 1990; Marczenko and Lobinski 1996). Mercury removal from anthropogenic sources, such as chlorine plants, pesticides and paints factories, becomes very important because from these sources it can contaminate water reservoirs and irrigation networks, and thus it may be retained by plants and different agents at various levels of the food chain.

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Viticulture represents an important agricultural practice in many countries (Komárek et al. 2010). The quality of irrigation water is an important variable defining the transportation of metals and other elements necessary for vine growth from soil toward the plant (Fabani et al. 2009). Irrigation with contaminated waters can seriously increase the probability of toxic metals uptake into vine plants. Irrigation waters can be contaminated from different sources like industrial effluents (Saleem-Saif et al. 2005), wastewaters (Wu and Cao 2010), and geological processes (Domagalski et al. 2004).

The metal uptake capacity of *Vitis vinifera* has been reported elsewhere (Leita et al. 1998; Todic et al. 2006; Chopin et al. 2008). Elevated heavy metal concentrations were found in xylem saps of vines showing a relatively high mobility within the plants (Leita et al. 1998). Chopin et al. (2008) studies showed that the differences between elements intake 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 (Todic et al. 2006; Spisso et al. 2013).

Plants respond to metal toxicity by initiating a wide range of cellular defense mechanisms (Thapa et al. 2012). These include immobilization, exclusion, and compartmentalization of metals; along with phytochelatins (PCs) synthesis (Sanitá di Toppi and Gabbrielli 1999). PCs are important molecules involved in the control of metal concentration in plants, fungus, and algae; being their main structure (γ-Glu-Cys)*n*-Gly with $n = 2$–11 (Kondo et al. 1985; Grill et al. 1987; Leopold and Gunther 1997; Gekeler et al. 1988). These peptides are produced in plants by γ-glutamylcysteine-dipeptidyl transpeptidase (phytochelatin synthase) as a consequence of exposure of plants to heavy metals, with glutathione (GSH) as a substrate. Several metal ions activate PCs synthesis, but Cd, Cu, Hg, and Zn are the most commonly studied because they induce a more intensive biological response in cells (Kondo et al. 1985). The role of PCs is toxic metals' coordination through –SH groups and metal–ion complex storing inside the cell's vacuole, avoiding any toxic effect of free metals in the cytosol (Zenk 1996). PCs accumulation has also been studied in different plants exposed to Hg contamination under controlled conditions (Sobrino-Plata et al. 2009; Sobrino-Plata et al. 2013). However, only a few studies have been devoted to specific Hg-PCs complexes determination in plants (Chen et al. 2009; Carrasco-Gil et al. 2011).

In order to determine PCs, chromatographic techniques coupled to several detectors have been used. Reversed-phase chromatography (RPC) with UV detection was applied with pre- and post-column derivatization strategies (Habeed 1972; de Knecht et al. 1994; Hirata et al. 2001; Chen et al. 2009). Fluorescence detection was also employed by derivatization with monobromobimane (mBBr), which forms fluorescent compounds with the thiol groups (mBBr-PCs) (Newton et al. 1981; Ahner et al. 1994; Rijstenbil and Wijnholds 1996; Minocha et al. 2008). RPC coupled to amperometric detection has been also used (Dago et al. 2011). In addition, metal-PCs complexes can be determined by combining capillary electrophoresis or liquid chromatography with atomic and molecular detectors. Moreover, mass spectrometry techniques have also been used to confirm the presence of PCs (Vacchina et al. 1999; Chen et al. 2009; Wood and Feldmann 2012).

This research is an attempt to increase the knowledge of Hg metabolism in vines. For the first time, it is described the determination of PCs complexed with Hg in vines. To this end, vine plants were irrigated with Hg contaminated waters under greenhouse conditions. After Hg-PCs extraction, a two-dimensional separation was applied: a first separation employing size exclusion chromatography (SEC) coupled to inductively coupled plasma (ICP MS) followed by a second Hg-PCs complexes separation and determination by RPC coupled to atomic fluorescence spectrometry (AFS). Finally, Hg-PCs complexes presence was confirmed by tandem mass spectrometry.

Materials and methods

Chemicals

Ultrapure water (18 MΩ cm^{-1}) was obtained from EASY pure (RF Barnstedt, IA, USA). Bovine serum albumin (66 kDa), alcohol dehydrogenase (150 kDa), β-amilase (200 kDa), thyroglobulin (669 kDa) and apoferrtin (443 kDa) from Sigma (St. Louis, MO, USA) were employed as standards for SEC. Phytochelatins standards (PC_2, PC_3 and PC_4) were obtained from Anaspec (USA). Nitric acid 65 % and trifluoroacetic acid (TFA)

were from Sigma-Aldrich. Mercury nitrate used in vine watering, sodium hydroxide, sodium monohydrogen phosphate, potassium dihydrogen phosphate and hydrogen peroxide were from Merck. Acetonitrile (ACN) and sodium dodecyl sulfate (SDS) were from Fisher Scientifics. Ethylenediaminetetraacetic acid (EDTA) (99 %) and tris (hidroximethyl) aminomethane (Tris; 99, 85 %) were from Across organics. Potassium persulfate ($K_2S_2O_8$) (99 %) was obtained from Fluka AG, (Switzerland). Sodium borohydride ($NaBH_4$) was obtained from Riedel-de Haën. Mercury standards were from Perkin-Elmer. Ammonium acetate was from Biopack.

Instrumentation

Microwave digestion was performed with a Milestone Start D microwave system (Sorisole, Italy), and with Milestone hermetically sealed 1 cm wall thickness polytetrafluoroethylene reactors (100 mL internal volume).

Mercury fluorescence measurements were carried out with an AFS, AI 3300, Aurora Instruments (Vancouver, BC, Canada). The apparatus was equipped with a two-channel peristaltic pump for the continuous fluorescence measurements. Hg hollow cathode lamp from Aurora Instruments (Vancouver, BC, Canada) was employed as Hg fluorescence excitation source. Samples and reagents involved in cold vapor generation (CV) were delivered by a Minipulse 3 peristaltic pump Gilson (Villiers-Le-Bell, France). Ultraviolet (UV) decomposition was achieved with a 400 W Hg vapor lamp (15 W G15T8 UV-C LONG LIFE high pressure Hg, PHILIPS) that ignited with a suitable starter and chock and surrounded by a 1.5 m PTFE tubing.

For extraction purposes, samples were dried in an Eppendorf Speed vac concentrator plus. A thermostatic ultrasonic washer (Cleanson) and Boeco Centrifuges U-320 R were also used.

ICP MS employed was a Perkin-Elmer Sciex, ELAN DRC-e (Thornhill, Canada). The argon (Ar) gas with a minimum purity of 99.996 % was supplied by Linde (Córdoba, Argentina). A high performance Teflon Nebulizer model PFA-ST, was coupled to a quartz cyclonic spray chamber with internal baffle and drain line cooled with the PC_3 system from ESI (Omaha, NE, USA).

Separations were performed with a Series 200, Perkin-Elmer (Thornhill, Canada) binary pump. The columns used were: for SEC, TSK gel G3000SW (7.5 × 300 mm × 10 μM), Tosoh Biosep; and for RPC, Zorbax SB-Aq C18-RP (1.6 × 150 mm, 5 μM) Agilent Technologies.

For confirmation of Hg-PCs structures, the mass spectrometry analyses were performed on a Quattro Premier™ XE Micromass MS Technologies triple quadrupole mass spectrometer with a ZSpray™ Electrospray ionization source (Waters, Milford, USA).

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.a.s.l. One-year-old plants of a selected clone of *V. vinifera L. cv. Malbec* were planted in 1.0 L plastic pots filled with 450 g of grape compost. Grape compost consists of 3 parts of pomace, 3 parts of loam and 2 parts of perlite (pH: 7.2; conductivity: 18.3 mΩ cm^{-1}; organic matter 9 %). They were grown in a greenhouse at temperatures ranging from 23 to 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. (2009) was adapted with modifications. A total of six plants were divided into two groups: control and samples. Control plants were watered only with 300 mL tap water, while sample plants were watered daily for three days with 300 mL of a 100 mg/L Hg solution in the form of mercury nitrate. The health of these plants was visually indifferent to Hg supplementation. After one week, samples of leaves, stems and roots were collected. They were immersed in an ultrasonic bath for a complete soil removal, washed with ultrapure water, and lyophilized. These plant organs were stored at −5 °C to prevent deterioration and enzyme activity that could cause species interconversion. The samples were used within few hours of storing.

Total Hg determinations

Determinations of total Hg in plant organs were performed by ICP MS after microwave assisted digestion. Thus 0.5 g of leaves were digested with 7 mL nitric acid (HNO_3), and 1 mL hydrogen

peroxide (H_2O_2); 0.3 g of stems with 8 mL of HNO_3 and 2 mL of H_2O_2 and 0.3 g of roots with 9 mL of HNO_3 and 1 mL of hydrofluoric acid. The digestion method for roots and stems consisted of two steps: first, one ramp of 20 min to 180 °C; and second, hold step of 10 min at a temperature of 180 °C. Maximum power (1,000 watts) was applied in both steps. For leaves digestion, a two-step method of ramp and hold was also used, each one of 10 min at a temperature of 200 °C and a power of 1,000 watts. Previous to digestion, roots were washed with an EDTA solution to remove the adsorbed mercury.

Phytochelatins extraction and determination

Prior chromatographic separations, extractions were carried out from vine samples (leaves, stems, roots) as follows: an extraction stage involving liquid nitrogen and a mortar followed by the addition of 2 mL of a 2 % (w/v) SDS–30 mM Tris-HCl buffer solution (pH:7) was performed. The extraction was completed after 2 h sonication and centrifugation at 5,000 rpm at 4 °C. Supernatants were collected and filtered through 0.22 μM filter (Osmonics®) prior injection (Mounicou et al. 2004). In order to evaluate the extraction efficiency, 15 and 3.5 μM of Hg and each PCs; respectively, were added to the samples, extracted under the above mentioned procedure and analyzed by LC–AFS. Quantitative results were achieved.

Determination of Hg-PCs complexes was performed by a two-dimensional chromatographic procedure. First, SEC was performed coupling the chromatographer to ICP MS. A 50 mM buffer ammonium acetate was employed as eluent being adequate for coupling with ICP MS, since its volatility do not generate deposits onto the 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. In order to determine the Hg associated to different protein or peptide fractions, 200 μL of each sample extracts (roots, stems and leaves) were injected and Hg and Sulfur (S) signals were monitored simultaneously. Sulfur determination was performed by means of its oxide detection, which is formed using oxygen as auxiliary gas. Thus sulfur acquires a mass/charge ratio of 48, being ICP MS adjusted for the simultaneous determination of Hg and SO^+ (Spisso et al. 2013). The working conditions were: dwell time; 15 ms; sweeps/reading, 40; carrier gas used was argon (Ar); 0.02 mL/min auxiliary gas used was oxygen (O_2); the mobile phase used was 50 mM ammonium acetate -with 5 % of methanol (v/v); at a flow rate of 0.7 mL/min in isocratic elution mode; sample injection was 200 μL. After the SEC approach, the fractions corresponding to Hg-PCs molecular weight were collected. These fractions were dried in Speed Vac and diluted to 1 mL with ultrapure water. The Hg-PCs complexes stability after the drying process was evaluated by standard addition, as described previously, with quantitative results. These fractions were then analyzed by RPC in a second dimension analysis. To this end, 100 μL of the collected fractions were injected to LC coupled to AFS. A gradient elution mode was employed, the mobile phases were A: 0.1 % (v/v) TFA in H_2O and B: ACN (0–50 % B 28 min; hold 50 % B 2 min and return to 100 % A in 5 min); flow rate was 1 mL/min. The reagents for CV generation were 0.1 % (w/v) $K_2S_2O_8$ in 30 % (v/v) HNO_3 and 0.5 % (w/v) $NaBH_4$ in 0.5 % (w/v) NaOH; flow rate were 1 and 2 mL/min respectively AFS conditions were: 253.7 nm Hg hollow cathode lamp wavelength; 300 V photo multiplayer power (PMT); 35 mA primary current; 100 °C oven and 200 mL/min carrier gas (Ar).

The choice of an AFS detector for reverse phase chromatography was mainly due to the fact that ICP MS does not support the introduction of large amounts of organic solvent, which is necessary for Hg-PCs complexes optimal separation.

In order to confirm the studied Hg-PCs complexes presence after RPC, mass analyses were performed on a triple quadrupole mass spectrometer configured with an electrospray ionization source operated in a positive (ES+) mode at a desolvation temperature 350 °C, with N_2 as nebulizer. The source was kept at a temperature of 150 °C. The capillary voltage was maintained at 3.0 kV and the extractor voltage was set at 3.0 kV. Optimization and detection was performed in a full scan mode via direct infusion (syringe pump) into the mass spectrometer of the Hg-PCs complexes (1 mg/L standard solutions for optimization purposes).

Results and discussion

Total Hg determinations

Total Hg determinations were performed in vine organs (roots, stems, and leaves) of control and

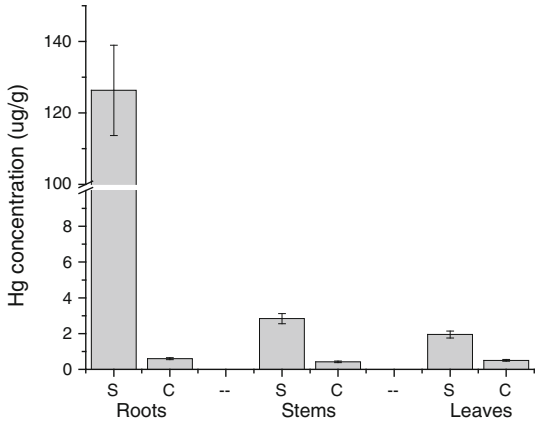

Fig. 1 Total Hg concentration into the different vine compartments evaluated for 100 mg/L of Hg^{2+} supplementation. Results are expressed as the mean ± standard deviation of 3 plants. *S* sample; *C* control

supplemented plants. In this work it was found that Hg concentrations in aerial organs were lower than roots in vines supplemented with Hg. However, these Hg levels are still higher than those found in control vines (0.42 ± 0.08 µg/g in stems, 0.5 ± 0.05 µg/g in leaves and 0.6 ± 0.05 µg/g in roots). It is frequent to find a certain degree of Hg accumulation in the leaves of control plants, as this metal is prone to volatize and accumulate in above-ground organs in plants cultivated in closed controlled environments. Similar behaviour was observed in Arabidopsis plants grown in hydroponics in a growth chamber (Sobrino-Plata et al. 2014).

Herein obtained results are shown in Fig. 1. The maximum Hg concentrations were found in roots with a value of 126.31 ± 4.96 µg/g. In stems Hg values were of 2.84 ± 0.84 µg/g. Leaves showed Hg concentrations of 1.95 ± 0.2 µg/g. Results are expressed as the mean ± the standard deviation of three samples. From the results, it was found that Hg values were higher in roots compared with stems and leaves, coincident with Hg values reported in our previous work (Spisso et al. 2013). Mercury undergoes almost complete sequestration in roots of vine plants, which prevents Hg accumulation in the aerial organs (leaves and stems). This observation has been described in different plants, in which a greater Hg concentration in roots than in other organs was observed (Iglesia-Turiño et al. 2006; Rellán-Álvarez et al. 2006; Chen et al. 2009; Carrasco-Gil et al. 2011; Chen and Yang 2012).

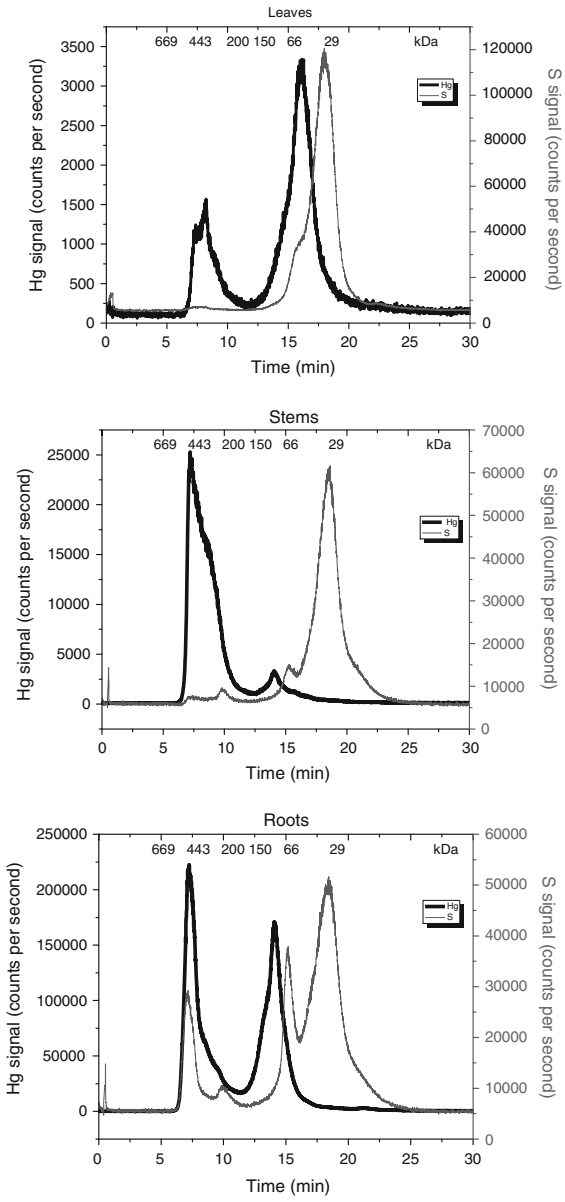

Fig. 2 SEC–ICP MS chromatograms of plant compartments. Molecular weight markers can be observed in the upper side of graphics. Standards and samples injection: 200 µL

Hg distribution and Hg associations with sulfur by SEC–ICP MS

The different Hg associations in plants were separated by SEC–ICP MS technique according to their molecular size. In this work, the column separation range used was wide: from 10 to 700 kDa. Hg and S signals were registered simultaneously by ICP MS. Sulfur was

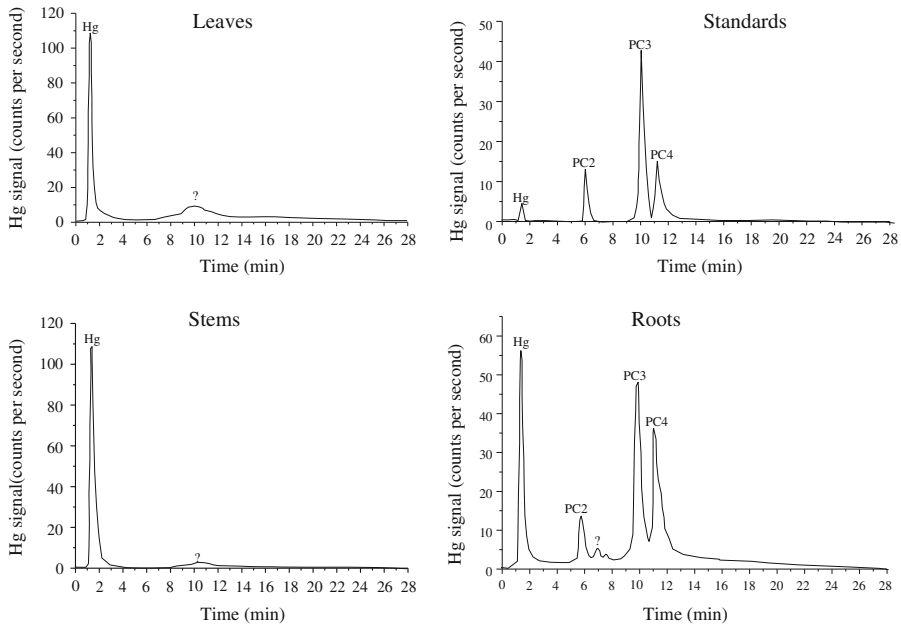

Fig. 3 Hg-PCs chromatograms by RPC-AFS analysis. Standards and samples injection: 100 µL. Standards concentrations: 15 and 3.5 µM for Hg and PCs respectively

monitored to evaluate possible Hg associations to proteins or peptides.

SEC chromatograms for roots, stems and leaves of vine plants are shown in Fig. 2. It can be observed that a Hg fraction appears in all vine organs in the range from 200 to 443 kDa. The estimated Hg concentrations in these fractions were 0.06 ; 0.984 and 6.133 µg/mL in leaves, stems, and roots; respectively. Only in roots, a sulfur fraction appeared associated to this molecular size fraction. A second Hg fraction appeared in the 29–100 kDa range, coincident with the S fractions in all the studied plant organs, indicating possible Hg associations of medium–low molecular weight. The estimated mercury concentrations were 0.160 ; 0.115 and 6.084 µg/mL in leaves, stems, and roots; respectively. These findings are in good agreement with separations reported in previous studies, which also described Hg distribution in two different molecular size fractions (Spisso et al. 2013).

After obtaining the SEC elution profile of Hg-S containing substances, medium–low molecular size fractions of the three vine organs were collected, as shown in Fig. 2. These fractions were chosen for collection considering parameters such as column separation capacity and molecular size that could be correspondent to Hg-PCs complexes. Fractions collected were from 12 to 19, 13 to 17, and from 11 to 17 min from leaves, stems, and roots chromatograms; respectively. These fractions were freeze dried and then diluted with ultrapure water for further Hg-PCs complexes analysis.

Hg-PCs determination by RP-HPLC-AFS

Hg-PCs complexes determination in the different vine organs was performed using a C18 reverse phase column for separation and determination. To this end AFS was employed as detection system being Hg the hetero-tagged atom. The chromatographic conditions were specified above in the text. Separation was completed employing ACN and TFA as described in bibliography for PCs separation (Chen et al. 2009; Dago et al. 2011).

Figure 3 shows the chromatogram obtained from PCs standard analysis in root, stem, and leaves SEC collected fractions. Hg-PCs complexes were prepared in phosphate buffer, with a concentration of 15 µM of Hg and 3.5 µM of each PC. Two hours incubation at room temperature was also applied to ensure complex formation. This concentration relationship between Hg and PCs ensured optimal Hg-PCs formation, with minimum concentration of Hg^{2+}, with a molar ratio of 2.14, 1.43 and 1.07 for PC_2, PC_3, and PC_4; respectively. As observed in the chromatogram of Fig. 3, four peaks corresponding to Hg^{2+} or unassociated Hg,

$Hg\text{-}PC_2$, $Hg\text{-}PC_3$ and $Hg\text{-}PC_4$, respectively, with baseline separation, were obtained.

Root chromatograms showed baseline separation between free Hg and different Hg-PCs complexes. PCs elution order was correspondent to those found in other published articles (de Knecht et al. 1994; Kawakami et al. 2006; Minocha et al. 2008). Elution times of Hg compounds were 77, 345, 593, and 660 s corresponding to free Hg^{2+}, $Hg\text{-}PC_2$, $Hg\text{-}PC_3$ and $Hg\text{-}PC_4$; respectively. The estimated mercury concentration of each fraction was 0.914 μg/mL for Hg, 0.309 μg/mL for $Hg\text{-}PC_2$, 1.272 μg/mL for $Hg\text{-}PC_3$, and 1.11 μg/mL for $Hg\text{-}PC_4$. Hg complexed with PCs corresponded to 1.43 % of the total Hg analyzed by SEC. Future studies will attempt to reveal other Hg associations since this time AFS was optimized for Hg-PCs determination. The above mentioned concentrations were calculated according to a calibration curve employing free Hg^{2+} and Hg-PCs standards injected in the LC-UV-CV-AFS system and are referred to the volume employed for dilution after Speed Vac drying (1 mL).

The Hg-PCs chromatograms of leaves and stems presented similarities, showing both a peak at 76 s corresponding to free Hg^{2+} and, also, a wide peak at 597 and 630 s in leaves and stems; respectively, with no correspondence with the investigated PCs.

From chromatograms analysis correspondent to the different studied vine organs, it was possible to conclude that Hg-PCs are present only in roots. This behavior can be explained considering that a higher Hg concentration favors a more effective Hg-PCs formation in roots. This behavior found in vines has also been observed in other plants where PCs were only identified in roots (Zeng et al. 2009).

Hg-PCs confirmation by ESI-MS/MS

After RPC-AFS analysis, fractions were collected as follows: one correspondent to the complex $Hg\text{-}PC_2$, collected at 250–450 s and a second fraction corresponding to $Hg\text{-}PC_3$ and $Hg\text{-}PC_4$ complexes collected at 500–900 s. These last two complexes were collected in the same fraction since their retention times were close. Collection times were broad than elution peaks to assure complexes gathering. Both fractions were dried down and then reconstituted separately with water for ESI-MS/MS analysis.

Table 1 Complex Hg-PCs

Hg-PC	Complex found	m/z
$Hg\text{-}PC_2$	$[HgPC_2 + H]^+$	740
	$[HgPC_2 - H_2O + H]^+$	722
$Hg\text{-}PC_3$	$[HgPC_3 + H]^+$	972
	$[HgPC_3 - H_2O + H]^+$	954
$Hg\text{-}PC_4$	$[HgPC_4 + H]^+$	1202
	$[HgPC_4 - H_2O + H]^+$	1184

The presence of the Hg-PCs complexes in roots was confirmed by determination of two ions for each studied complex, which are shown in Table 1. These Hg-PCs complexes are: m/z: 740 ($Hg\text{-}PC_2$), 972 ($Hg\text{-}PC_3$), 1202 ($Hg\text{-}PC_4$), have been also reported by Chen et al. (2009). In this way Hg-PCs formation in vines under Hg stress was correspondent with Sobrino-Plata et al. (2013) research, where these complexes formation were determined in *Arabidopsis thaliana*.

Conclusion

Vine plants react to stress generated by heavy metals, producing different phytochelatins with metal complexing function. In this sense, the stress induced in vine plants through irrigation with Hg contaminated waters lead to the formation of $Hg\text{-}PC_2$, $Hg\text{-}PC_3$ and $Hg\text{-}PC_4$ complexes.

As mentioned, higher Hg concentration levels in roots rather than in aerial organs (stems and leaves) were found. This could probably be explained by the way Hg mobilizes across the plant. This metal enters through the roots, where it is complexed by phytochelatins and thus cannot be mobilized to aerial organs. This sequestration process might be interesting considering it as an impediment for Hg to reach the edible vine organs.

A first approach by SEC–ICP MS analysis showed that Hg was distributed in plant organs in two different fractions, high and medium–low molecular weight; respectively, being this last one important since Hg-PCs compounds can be distributed within this fraction. In addition sulfur monitoring confirmed peptide presence in this medium–low molecular weight portion. A second strategy by RPC analysis showed a baseline separation of $Hg\text{-}PC_2$, $Hg\text{-}PC_3$, and $Hg\text{-}PC_4$ complexes in the medium–low molecular weight

fractions collected from SEC. Such complexes were only found in vine roots. The presence of these compounds was verified by ESI-MS/MS analysis, encompassing RPC results. Overall the proposed technique allowed a successful separation, isolation, and identification of Hg-PCs complexes.

Thus Hg-PCs complexes have been studied and determined in vine plants for the first time achieving a breakthrough in the elucidation of vine defense mechanism against heavy metals from irrigation water. This work acquires significance considering vines economic importance as grapes provider for wines production. Pollution of irrigation waters from different sources is a matter of great concern since affects the safety and quality of wines.

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