Experimental assessment of the daily exchange of atmospheric mercury in *Epipremnum aureum*

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Abstract Mercury (Hg) exchange at the plant leaf–atmosphere interface is an important issue when considering vegetation as a sink or source of this global pollutant. The aim of the study described here was to clarify this process by studying Hg exchange under laboratory conditions with a plant model, namely *Epipremnum aureum*. The desorption and absorption processes were studied under similar conditions in natural daylight. Hg exchange was measured at the foliar surface, and micrometeorological parameters and stomatal conductance were assessed. The results of the Hg exchange study showed different rhythms for the two processes, i.e. desorption ($14\text{--}196\text{ ng m}^{-2}\text{ day}^{-1}$) was slower than absorption ($170\text{--}1341\text{ ng m}^{-2}\text{ day}^{-1}$). The daily cycle was more complex in the desorption process, with a maximum when stomatal conductance was high but also with high values during nocturnal hours and a trend to absorption in the mornings. The daily absorption cycles were relatively simple, with values

that coincided with positive stomatal conductance values and null values during nocturnal hours. The main factors involved in desorption were stomatal conductance and temperature, but other factors may need to be considered. The absorption process only involved total gaseous Hg, stomatal conductance and relative humidity. A net balance of the two experiments provided data on the amount of Hg transferred per unit leaf area (167 ng m^{-2} for desorption and 9213 ng m^{-2} for absorption), which implies total amounts of 23 ng of Hg desorbed and 1280 ng absorbed during the whole experiment. Finally, the reversible/non-reversible nature of the Hg exchange process must be reconsidered bearing in mind that Hg within the leaf can be emitted if changes in ambient conditions are appropriate to favour this process.

Keywords Mercury · Plant–atmosphere · Stomatal conductance · *Epipremnum aureum* · Gas exchange

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Introduction and background

Mercury (Hg) is an element that is present in the environment, and it exists in the three states of matter: liquid (rarely), solid and gas. The ability to transform this element from the solid or liquid to the gaseous state leads to very complex global cycling processes, in which the important aspects to consider are flows

between different environmental compartments (terrestrial, freshwater, oceans and atmosphere) and transformations from one state to another (Beckers and Rinklebe 2017). Once Hg has been emitted by a source, either anthropogenic or natural, it undergoes transformations between Hg^0 – Hg^{2+} –particulate Hg in the atmosphere, or between Hg^0 – Hg^{2+} – MeHg^+ in terrestrial or aquatic environments. In the terrestrial environment, these Hg compounds are preferentially found in soils, bound to organic matter, as a product of atmospheric deposition (Si and Ariya 2018). Vegetation plays an important role in the Hg exchange process between the soil and the atmosphere, with Hg acquired from the soil via the roots (Liu et al. 2017; Ahammad et al. 2018) and from the atmosphere via the leaves (Kowalski and Frankowski 2016; Zheng et al. 2017; Barquero et al. 2019).

Plants act as a temporary sink for Hg, and they provide a transfer pathway from the soil to the atmosphere or from the atmosphere to the soil via litterfall. It has been established in numerous studies that direct Hg uptake by plants from the soil is minor compared to the Hg emission/deposition direct from the atmosphere (Assad et al. 2016; Kowalski and Frankowski 2016; Zheng et al. 2017; Higuera et al. 2016; Barquero et al. 2019; Esbrí et al. 2018). A great deal of effort has been devoted to clarifying gaseous elemental Hg (GEM) fluxes from the soil to the atmosphere, with a global cycle of net emissions reported in warm seasons and negligible flux in colder periods (Sommar et al. 2012; Demers et al. 2013). In terrestrial ecosystems, it is necessary to consider the vegetation given that it can act as a source or sink depending on the micrometeorological conditions and plant community composition (Molina et al. 2006; Campos et al. 2018; Bash and Miller 2007; Converse et al. 2010; Higuera et al. 2012; Amorós et al. 2014). Eckley et al. (2016) found that vegetation seems to have an impact on soil–atmosphere Hg fluxes due to a reduction in solar radiation reaching the soil and to the direct uptake of Hg emitted by the soil. However, this direct uptake involves a continuous exchange of Hg and it is necessary to clarify the cycling of Hg fluxes. The approaches employed to make these canopy flux measurements include micrometeorological methods (mainly Bowen and Eddy covariance monitoring stations) to monitor a complete forest mass (Yu et al. 2018), the use of a dynamic flux chamber to study the flux of a plant specimen in situ (Fu et al. 2010;

Millhollen et al. 2006) and controlled conditions in the laboratory (Stamenkovic and Gustin 2009).

Obrist et al. (2012) stated that Hg flux at the terrestrial–atmosphere interface is not well understood, especially when factors like the absence of GEM (background areas) or the existence of vegetation have a relevant impact in the area of study. Plants represent the dominant pathway for Hg deposition in these environments, mainly due to litterfall but also as a result of throughfall, with 70–85% of total Hg deposition reached in a forest environment (Wang et al. 2016). Litterfall can be enriched in Hg direct from the atmosphere by wet deposition (Zheng et al. 2016) or dry deposition (Witt et al. 2009), but, surprisingly, most of the Hg in litterfall comes from woody biomass—at a level of some 70%—and not from foliage (Obrist et al. 2018; Yang et al. 2017). The acquisition pathway for this Hg in plant tissue (wood and leaves) is mostly atmospheric and occurs by two routes, namely through the stomata in leaves and through cuticle/cortex in the leaves/trunk. Some authors have estimated that Hg acquisition via stomata or the cuticle can lead to differences in reversibility, but this is not well established. Stamenkovic and Gustin (2009) estimated that Hg leaf uptake has both a reversible component and a non-reversible component depending on the acquisition route, thus implying the oxidation of Hg^0 taken up by the leaf. Rutter et al. (2011) estimated that 96% of Hg uptake goes to the interior of the leaf and this involves both the exchangeable Hg fraction and the non-exchangeable fraction. These conclusions were confirmed by Naharro et al. (2018), who estimated that non-reversible exchange is the minor component for olive trees in a Hg-contaminated site.

The objective of the study reported here was to evaluate the daily Hg cycle in plants in contrasting situations of Hg absorption/desorption. The aim was to estimate the proportions of reversible/non-reversible components in the Hg uptake process. The plant model selected for the acquisition of experimental data was *Epipremnum aureum*. This plant was selected due its ubiquity in tropical environments in Asia and the Pacific islands, the fact that it is a common houseplant around the world, its high foliar surface, its known high Hg uptake capacity and because it has proven to be adapted to high gaseous Hg environments in the ‘Instituto de Geología Aplicada’ (IGeA) sample storage unit.

Experimental section

The experimental design involved the isolation of three branches of *Epipremnum aureum* in a Tedlar bag equipped with one inlet line and one outlet line (Fig. 1). The Tedlar material was chosen because it is the most widely used type of bag for taking samples of gases due to its very low gas permeability and its low capacity to produce cross-contamination problems.

Total gaseous Hg in the interior of the bag was monitored by atomic fluorescence spectrometry, using a Tekran 2537B device coupled through the outlet line. A sampling time of 5 min and a flow rate of 1 litre per minute were chosen to complete the air renewal in the bag within a short period of time (less than 10 min). The volume of air contained in the bag was 8.67 L, as calculated indirectly from the dimensions of the bag. In order to ensure that the bag was completely

Fig. 1 Experimental set-up for the desorption (a) and absorption (b) experiments

inflated during the experiment, a commercial air pump was used to insufflate the same quantity of ambient air as extracted from the bag by the measurement device. Inlet air coming from the room was monitored by atomic absorption spectrometry using a Lumex RA-915M device to ensure analysis of the same air that entered the bag. These two analytical devices have proven to be compatible for the simultaneous measurement of the same phenomenon during an inter-comparison analysis conducted in the IGeA laboratory in collaboration with the Carlos III Health Institute, for which the two teams showed a compatibility index (ISO/IEC 1997) of less than 1 (Fernández-Patier and Ramos-Díaz 2011). The quality control of the measurements from the Tekran equipment was confirmed by checking the calibration with an internal permeation source every 3 days and recalibrating every week. The quality control for the Lumex device involved performing a test before each measurement with an internal source of Hg vapour, and this monitored the drift of the equipment; this drift was always kept below 3%, although the manufacturer states a deviation of less than 20%.

Stomatal conductance measurements on *Epipremnum aureum* were performed by means of a leaf steady-state diffusion porometer using a Decagon device (model SC-1). Manual measurements were performed hourly for 48 h, with other plant species included for comparative purposes (*Plectranthus*, *Begonia acutifolia*, *Begonia coccinea*, *Vitis vinifera*, *Laurus nobilis*, *Citrus sinensis* and *Citrus limon*). Each data consisted of a single measurement carried out on young and fully developed leaves of the branch, and the values are expressed in $\text{mmol m}^{-2} \text{s}^{-1}$. Evergreen leaves are usually less effective than deciduous ones in terms of photosynthesis intensity, but in many cases this discrepancy is balanced by their longevity. There is an inverse relationship between the age of the leaf and its photosynthetic activity (Reich et al. 1992) and its capacity to accumulate contaminants (Higueras et al. 2012). The net photosynthesis in woody species ranges from 3 to 12 $\mu\text{mol CO}_2 \text{ m}^{-1} \text{s}^{-1}$, and there is a large difference between shade-adapted species and those adapted to sun exposure (15–60 $\text{mg CO}_2 \text{ g}^{-1} \text{h}^{-1}$) (Cronan 2018). In order to ensure reproducibility of the measurements, two identical plant specimens (*Begonia acutifolia*) were monitored and similar cycles were obtained ($R^2 = 0.78$), although plants

were oriented differently with respect to solar radiation.

Other data acquired during the desorption/absorption experiments were ambient temperature, relative humidity, solar radiation, atmospheric pressure, dew point and evapotranspiration. The measurements were taken inside and outside the pump inlet box by portable meters with an EL-USB-2 datalogger (model Lascar), which is capable of storing a large amount of data continuously. Solar radiation, dew point and evapotranspiration were measured using an automatic Davis Vantage Pro micrometeorological station with a console and datalogger for time periods of 15-min data storage.

Three branches were introduced into the Tedlar bag, and the total leaf surface area was 0.139 m^2 , with leaf surface areas of 0.039 m^2 , 0.061 m^2 and 0.039 m^2 for the three branches. Calculation of the leaf surface was performed by 1:1 scale planimetry using the silhouette of the leaves. Signs of leaf damage were not observed during the experiments, and the increment in leaf surface was restricted to 0.002 m^2 due to the natural development of branches with new leaves. Gaseous Hg exchange by the plant was calculated using the following equations:

$$\text{TGM} = \text{TekranHg} - \text{LumexHg}$$

$$\text{BagHg} = \text{TGM} \times \text{Bagvolume}$$

$$\text{ExchangeHg} = \text{BagHg}/\text{leaf surface}$$

where TGM is the total gaseous Hg concentration expressed in ng m^{-3} ; TekranHg is the TGM inside the bag measured by the Tekran device, expressed in ng m^{-3} ; LumexHg is the TGM outside the bag measured by the Lumex device, expressed in ng m^{-3} ; BagHg is the total amount of TGM in the bag expressed in ng; Bagvolume is the volume of air in the Tedlar bag, expressed in m^{-3} ; and ExchangeHg is the TGM exchange by leaf, expressed in ng m^{-2} , in a determined period of time, normally $\text{ng m}^{-2} \text{h}^{-1}$ in daily cycles or $\text{ng m}^{-2} \text{day}^{-1}$ over the period of the experiment.

Two different experiments were carried out with the *Epipremnum aureum* isolated in the Tedlar bag: a desorption experiment from 27 April 2018 to 1 June 2018 and an absorption experiment from 1 June 2018 to 25 June 2018. Prior to beginning the desorption experiment, the specimen of *Epipremnum aureum* was

maintained in a TGM-rich atmosphere (250 ng m^{-3} on average) in a core sample storage unit at the IGeA facility in Almadén (Spain). The exposure time was 3 months, and the THg concentration reached 245 ng g^{-1} in leaf (average, wet weight). In this experimental phase, the plant was moved from the Almadén IGeA facility to a laboratory in Ciudad Real (Higher Technical School of Agricultural Engineers, 100 km away from Almadén) to be stored under conditions with low atmospheric gaseous Hg ($2\text{--}3 \text{ ng m}^{-3}$ in the laboratory). The plant *Epipremnum aureum* was placed in a room in an appropriate orientation with respect to the window so that it did not receive direct sunlight at any time. The desorption experiment involved monitoring the Hg exchange between leaf and the atmosphere within the bag, without any change in the experimental set-up. In contrast, the absorption experiment required some modifications: the main change was the allocation of a gaseous Hg source inside a cardboard box, which was totally opaque, and monitoring with the Lumex equipment. Small pieces of cinnabar samples rich in metallic Hg were placed inside the box as gaseous Hg sources, with an increasing number of samples to increase the TGM concentration inside the Tedlar bag (Fig. 1). Four experiments were performed, each with a duration of one week: three with natural solar radiation with Hg concentrations inside the bag of 24, 37 and 160 ng m^{-3} , respectively, and the last under dark conditions with an Hg concentration inside the bag of 160 ng m^{-3} . The concentrations were selected as low, medium and high exposure rates in Almadén city (Esbrí et al. 2016).

Results and discussion

Stomatal conductance

The stomatal conductance daily cycle clearly appears to be controlled by the cycle of solar radiation (Fig. 2a), with an increase in stomatal conductance from sunrise at 07:00 to a maximum at 10:00, a second moderate increase during the period 14:00–15:00, a slight increase at 20:00 and a decrease with nightfall to zero conductance at 22:00. Daily cycles for other shrub plants (Fig. 2b, c) showed a similar pattern for two specimens of *Begonia acutifolia*, while the maxima for *Plectranthus*, *Vitis vinifera* and *Begonia*

coccinea were reached later at 13:00 and 17:00. This behaviour—with the maximum in the middle of the day—is coincident with the behaviour of the tree species considered, i.e. *Citrus limon*, *Citrus sinensis* and *Laurus nobilis*. A single Pearson correlation study of the daily cycle of *Epipremnum aureum* with temperature, relative humidity and solar radiation showed the absence of significant correlations, but *Begonia coccinea* gave an R^2 value of 0.70 with relative humidity, *Laurus nobilis* an R^2 of 0.78 with solar radiation and 0.71 with temperature and *Citrus sinensis* an R^2 of 0.71 with temperature. This lack of correlations suggests that the response of stomata to environmental and physiological factors cannot be explained by micrometeorological parameters alone and that some other factors, such as CO_2 concentration, guard cell and epidermal turgor, may need to be considered (Buckley and Mott 2002; Mencuccini et al. 2000; Franks et al. 2001).

Stomatal conductance values for *Epipremnum aureum* are in the range $3.7\text{--}5.5 \text{ mmol m}^{-2} \text{ s}^{-1}$, while all other studied plants showed higher levels (from $10.1 \text{ mmol m}^{-2} \text{ s}^{-1}$ for *Begonia acutifolia* to $43.5 \text{ mmol m}^{-2} \text{ s}^{-1}$ for *Vitis vinifera*). These figures are low in comparison with others reported in the recent literature (see Lombardozzi et al. 2017). Segev et al. (2015) provided contrasting data about stomata: the number of stomata per unit leaf surface of *Epipremnum aureum* was the lowest (8), while other plants showed higher values, e.g. *Hedera spp.* (26), *Spinacia oleracea* (13), *Vinca minor* (13) and *Rhododendron spp.* (9). However, *Epipremnum aureum* gave the highest values for other parameters like stomatal index and net rate of photosynthesis.

It should be emphasized that *Epipremnum aureum* was the only plant monitored in this study that showed night values of zero—the other plants gave values below $5 \text{ mmol m}^{-2} \text{ s}^{-1}$ (*Plectranthus*, *Begonia coccinea*, *Citrus limon*, *Citrus sinensis* and *Laurus nobilis*) or up to $5 \text{ mmol m}^{-2} \text{ s}^{-1}$ (*Begonia acutifolia* and *Vitis vinifera*). This topic has recently gained increasing interest in the scientific literature due to the implications on global climate change and the ability of plants to sequester CO_2 during night-time (Lombardozzi et al. 2017). The stomata of the selected plant species will be completely closed by night, so if leaves uptake/emit Hg at night it will mean that this absorption/desorption should occur through cuticula and not through the photosynthesis apparatus.

◀ **Fig. 2** Stomatal conductance daily graph for *Epipremnum aureum* (a) during two cycles of 24 h and for other common plant species (b) and trees (c) during a cycle of 24 h

Desorption experiment

During the first week (Fig. 3a), the general trend was a decrease in desorption, starting from an initial situation in which the emission occurred throughout the day, with a maximum value of $0.8 \text{ ng m}^{-2} \text{ day}^{-1}$ during the first day and decreasing further in terms of daily desorption to an average of $0.2 \text{ ng m}^{-2} \text{ day}^{-1}$ on the seventh day. The daily cycle continued to show net desorption as of April 28, although desorption showed a sharp decline towards net absorption between 05:00

and 06:00 and a maximum desorption between 13:30 and 14:30. Watering of the plant on April 30 led to a marked increase in relative humidity in the laboratory and a marked decrease in the Hg concentrations both inside and outside the bag, with this change being more pronounced outside the bag.

If we focus on the 29 and 30 April (Fig. 3b), it can be seen that the daily desorption minimum is now at 05:00–06:00 and the daily desorption maximum is between 13:30 and 14:30. The effect that irrigation has on the daily maximum is to decrease absorption values, a finding that can be explained in terms of the rise of wet deposition due to the rise in relative humidity in the confined atmosphere of the Tedlar bag. On both days, there was also a moderate rise in

Fig. 3 Graphs of Hg flux during the first week of the desorption experiment (a) and a detail of the graph 2 days after the start of the experiment (b)

desorption in the period 20:30–22:30. At this time, the solar radiation is zero, the photosynthesis process is interrupted, the stomata close and the Hg exchange is falling to zero—even when a temperature drop occurs. These factors are not sufficient to explain the moderate increase in desorption, and the trend over the 2 days is for a decrease; the night-time minimum, the mid-day high and the moderate increase at dusk are lower on April 30 when compared to the previous day.

During the second week, it was observed that 10 days after the end of the graph shown in Fig. 3b, the desorption pattern and magnitude remained similar (Fig. 4a). In this case, the sharp decline occurred in the period 04:00–06:00, the maximum was again recorded in the period 13:30–14:30 and a final increase in desorption was observed at the end of the day, with a maximum in this case in the last hour of the day. The most marked difference on this day was observed in the period 12:00–13:00, which was a sharp decline to net absorption of Hg. The most obvious change in the monitored micrometeorological conditions occurs in relative humidity levels, showing a decrement which

coincided with the change from desorption to absorption. Relative humidity can be involved in the photosynthesis cycle of the plant since the exchange of liquids is estimated to be greater than the exchange of gases (Beer et al. 2009). Furthermore, it has been reported that very low relative humidity levels can decrease the respiration rate and even close the stomata to avoid excessive water loss.

The same daily cycle as above is shown in Fig. 4b but with fewer nocturnal variations in relative humidity (and values lower than those shown in Fig. 4a) and with similar variations in temperature and irrigation (17:00). It can be seen from the graph that a decrease in desorption at dawn (04:00–06:00) no longer occurs, so it is possible that a factor involved in this decline was relative humidity, which was more stable and lower on the night corresponding to the data shown in Fig. 4b. However, the decline in desorption during the period 12:00–13:00 continues to occur, with similar conditions of temperature and relative humidity. The maximum in this case again occurs in the period 13:30–14:30, and this coincides with the cycle of solar radiation

Fig. 4 Daily Hg desorption profile 12 days (a), 14 days (b) and 26 days (c) after the beginning of the desorption experiment and evolution of Hg flux during the desorption experiment (d). RH relative humidity, SR solar radiation. Units: RH (%), SR (W m^{-2})

light in the laboratory, especially with respect to the decrease in the intensity of solar radiation. (The increase in desorption also coincides with the rise in temperature.) These findings indicate that the photosynthesis cycle and the stomatal conductance seem to be the main factors that contribute to the observed desorption maximum. The secondary rise at nightfall is more pronounced and higher desorption values are observed, which coincide with irrigation of the plant and the lower relative humidity values in this period. From the observations outlined above, it can be suggested that a lower relative humidity could be a factor that activates the desorption of Hg during the night, both at the beginning and at the end of the day, while the maximum desorption appears to be controlled by the diurnal cycle of sunlight. The peak absorption always coincides with the first few hours of the day, when stomata are open and CO₂ fixation is maximum; besides, in this period absorption is more important than it was 10 days before (Fig. 3b).

Over the following 10 days (Fig. 4c), a marked decrease was observed in the rate of desorption from the leaves of *Epipremnum aureum*, to such an extent that desorption and absorption appeared to be reaching equilibrium. The minimum and maximum trends for the net desorption period (Fig. 2) were no longer maintained in this stage of equilibrium: a decline was not observed in the early morning and neither was the maximum at 13:30–14:30, with this time period replaced by a trend towards absorption during the first period of active stomatal conductance and desorption in the second part of the photosynthesis cycle. Before and after the stomata are open, the general trend is towards desorption. This situation is similar to that described by Stamenkovic and Gustin (2009), who explained the episodes of desorption (emission) as being due to the Hg that was adsorbed in the cuticle of the leaf.

The evolution of the desorption and absorption rates over the 35 days of the desorption experiment is represented in Fig. 4d. The desorption maximum occurred at the beginning of the experiment, with zero absorption rates for almost 20 days. In the last phase of the experiment, the leaves began to reach equilibrium with the Hg in the atmosphere in the bag and a continuous absorption–desorption exchange was established. The lack of gradation or linearity in the decrease in desorption from the beginning of the experiment is remarkable. This phenomenon could be

due to a failure in the datalogger, i.e. missing data, which produces two straight lines in the graph that could, in fact, be oscillations like those observed at the end of the graph. The amount of Hg per unit leaf area for the net balance was calculated from this graph and a value of 167 ng m⁻² was obtained, which represents a total amount of 23.21 ng Hg emitted during the whole experiment.

Although a significant linear correlation was not found between Hg exchange and micrometeorological parameters, a multivariate analysis did show a relationship with temperature (72.89 of similitude in a dendrogram with Ward linkage) but not with relative humidity, dew point or solar radiation (similitudes of – 30.35).

Absorption experiment

The results of the four absorption experiments are summarized in Fig. 5a. The most obvious findings are the absence of desorption periods in a daily cycle and the coincidence of absorption values with stomatal conductance up to zero. Furthermore, it is worth highlighting the marked similarity of the desorption profiles of experiments at low gaseous Hg rates (24 ng m⁻³ and 37 ng m⁻³) obtained with different Hg vapour sources (metallic Hg for 24 ng m⁻³ and cinnabar for 37 ng m⁻³). The concentration of TGM in the atmosphere in which the plant is kept has been described as a key factor to explain plant Hg uptake. However, the data obtained in this experiment suggest that minor differences in this factor do not influence the Hg acquisition process, so other factors like stomatal conductance rates or CO₂ concentrations, amongst others, may play important roles in this process. On the other hand, when plant exposure to Hg reaches very high levels of gaseous Hg (160 ng m⁻³), the profile is significantly wider. These three profiles are very similar in terms of daily evolution, with Hg absorption beginning with the first light of day and the absorption cycle concluding when the solar light cycle ends. It is evident that the photosynthesis cycle of *Epipremnum aureum* (Fig. 2) is the main factor in the distribution of maximum and minimum values in the absorption cycle of the plant, although this is not as clear in terms of the absolute values of absorbed Hg. This relationship is even more evident if one considers the difference between the cycle at 160 ng m⁻³ Hg in light and in total darkness. When the plant does not

Fig. 5 Absorption profiles for *Epipremnum aureum* at different rates of TGM exposure (a) and relationship between TGM inside the bag and Hg uptake by the plant (b)

detect sunlight at the beginning of the day, the absorption cycle does not begin but the plant absorbs and desorbs small concentrations at intervals dependent on other factors (temperature, relative humidity, dew point, etc.).

The total amounts of Hg absorbed per unit leaf surface over 24 h in the four experiments were on average $269.5 \text{ ng m}^{-2} \text{ day}^{-1}$ (TGM = 24 ng m^{-3}), $352.5 \text{ ng m}^{-2} \text{ day}^{-1}$ (TGM = 37 ng m^{-3}), $1079.6 \text{ ng m}^{-2} \text{ day}^{-1}$ (TGM = 160 ng m^{-3}) and $49.2 \text{ ng m}^{-2} \text{ day}^{-1}$ (TGM = 160 ng m^{-3}) in total darkness. At the end of these four different experiments, Hg plant uptake was estimated to be 9213 ng m^{-2} , i.e. a total of 1280 ng for the three branches exposed within the bag.

A significant correlation was found between TGM inside the bag and Hg exchange (a balance of Hg

absorbed and emitted) by the plant (Fig. 5b). Hg exchange values corresponding to low TGM levels were located above the trend line, which suggests that Hg uptake capacity is higher at low TGM levels and/or Hg uptake capacity has a gradual limitation in relation to increasing TGM levels.

Although a significant linear correlation does not exist between Hg exchange and micrometeorological parameters, a multivariate analysis displayed a weak relationship with relative humidity and dew point (similitude of 46.17 in a dendrogram with Ward linkage) but no relationship with temperature or solar radiation (similitudes of -50.12).

Discussion

Epipremnum aureum leaves showed different patterns in the daily desorption–absorption cycles, and desorption ($14\text{--}196\text{ ng m}^{-2}\text{ day}^{-1}$) was a slower process than absorption ($170\text{--}1341\text{ ng m}^{-2}\text{ day}^{-1}$). As reported by Stamenkovic and Gustin (2009) for other plant species, the Hg concentration in air has a significant effect on the leaf–atmosphere Hg flux, which increases proportionally with the presence of TGM, from pristine levels in the desorption experiment to higher levels of 160 ng m^{-3} in the third and fourth absorption experiments. Kothny (1973) described for the first time a pulse of Hg emission from vegetation after sunrise, and this idea was confirmed by other authors, who described diurnal net Hg emission frames (Yu et al. 2018; Stamenkovic and Gustin 2009, amongst others). The work reported here provides the first example of data on the daily desorption cycle after an intense absorption time frame using a common ornamental plant (*Epipremnum aureum*) previously spiked with Hg directly taken up from a naturally enriched atmosphere. These desorption cycles showed maxima that coincided with maximum solar radiation and active stomatal conductance but, surprisingly, showed other minor desorption periods under nocturnal conditions and a tendency to absorption values after sunrise. These cycles appear to be more complex than others described for canopy Hg emission (Yu et al. 2018; Luo et al. 2016), although the photosynthesis cycle was the simplest of all plant factors studied. It is important to note that the data presented here correspond not to emission in a continuous exchange context, but to desorption under conditions of previous high Hg uptake in leaves and very low TGM in the atmosphere. The existence of nocturnal desorption periods indicates that pathways other than the stomatal route may be acting, thus favouring Hg emission from leaf tissue. Rutter et al. (2011) estimated that Hg uptake goes directly to the interior of the leaf (96%) and only a small quantity (4%) remains in the cuticula of the leaf. These authors considered that 96% of Hg will remain in the leaf as non-desorbable Hg and only a small quantity (4%) should be emitted to the atmosphere under favourable conditions. It is shown in Fig. 3a that the emission of this surficial Hg can occur in the first stage of the Hg desorption cycle and subsequently the desorbed Hg can come either from the cuticula or from the interior

of the leaf, mostly as Hg^0 but also as Hg^{2+} . These data are consistent with the previous results reported by Naharro et al. (2018), who stated that almost 37% of Hg previously taken up by olive trees can be desorbed in a pristine area—a proportion higher than the minor fraction described by Rutter et al. (2011). These data limit the non-reversible character of the Hg uptake process, and this suggests that a fraction of the ‘immobilized’ Hg in the interior of the leaf could be emitted if TGM levels in the atmosphere decrease dramatically (Naharro et al. 2018). This ‘immobilized’ Hg fraction in the interior of the leaf was not only in an oxidized state but was also metabolized Hg bound to biothiols or proteins (cysteine complexes; Carrasco-Gil et al. 2013). Thus, metabolic processes can liberate these compounds and favour Hg re-emission. For instance, some of these Hg species can be bound to plasma membrane proteins such as aquaporins (Zhang and Tyerman 1999), they can be involved in water movement between cells, and they are susceptible to acting as a transport route for Hg to the exterior of the leaf. Another pathway through which Hg can be emitted involves the fraction bound to phytochelatin or cysteine, which can be involved in long-distance metal transport in the interior of the plant, e.g. from root to shoot and vice versa (Gong et al. 2003), and this acts as a mobilizing process. The statistical relationship between desorption data and temperature confirms the role of these transport pathways in Hg re-emission. In any case, the exit route from the leaf during nocturnal periods, in which the stomata are completely closed (as shown in this work), remains unexplained.

On the other hand, the absorption process in *Epipremnum aureum* showed a typical daily cycle for all of the conditions investigated, with Hg uptake varying during daily hours in line with solar radiation and stomatal conductance, and with null Hg flux during nocturnal hours, as reported by other authors for different plant species (Leonard et al. 1998; Ericksen et al. 2003; Stamenkovic and Gustin 2009). The key factors that affect this absorption process are TGM in the ambient air and stomatal conductance. The first factor acts as a regulator of the proportional uptake capacity, which is higher under low TGM conditions and decreases as TGM levels increase. The implication of this finding is that Hg uptake capacity may tend to a maximum limit depending on the plant species. The second factor is also a limiting one and

constitutes the unique pathway through which Hg enters the leaf tissue. Relative humidity could be a secondary factor as TGM levels are modified by wet deposition and stomatal conductance through changes in water equilibria between plant cells and the ambient air. It is important to note that nocturnal absorption was not observed, thus ruling out absorption through cuticula in *Epipremnum aureum*.

The combination of the two processes discussed above, i.e. absorption and desorption in plant species (*Epipremnum aureum*), offers for the first time a complete overview of the possibilities for plants to act as a sink or source of atmospheric Hg if ambient conditions change sufficiently. The absorption process appears to be faster than desorption, and this involves stomatal conductance, TGM levels and relative humidity as limiting factors. In contrast, the desorption process seems to be more complex and involves not only stomatal conductance and temperature but also CO₂ concentration, guard cell, epidermal turgor and Hg speciation inside the leaf, amongst other factors. The reversible/non-reversible nature of the process described in the scientific literature must be reconsidered, especially bearing in mind that Hg in the leaf interior can be emitted if the changes in ambient conditions are sufficient to favour this process. The most important change that can produce this Hg emission is a decline in the TGM level. This effect could be relevant in the context of climate change and the reduction in anthropogenic Hg emissions, for instance, after the reclamation of polluted areas, and it is probably due to the slowing down of the decrease in TGM levels when the desorption rates of vegetation increase as TGM declines.

Conclusions

The main findings of the work described above are as follows:

- Stomatal conductance daily cycles differ as a function of plant species, with nocturnal positive values in common plants except for the plant model employed in this study (*Epipremnum aureum*).
- Mercury can be desorbed under laboratory conditions at rates of 14–196 ng m⁻² day⁻¹ when TGM levels fall to pristine levels, with cycles showing

maximum Hg exchange values that coincide with higher stomatal conductance and also during nocturnal hours, but with a tendency towards absorption values during morning hours.

- The absorption of Hg under laboratory conditions reaches values of 170–1341 ng m⁻² day⁻¹, with simple cycles of daily absorption periods that coincide with positive stomatal conductance. The total amounts of Hg absorbed per leaf surface in a period of 24 h were on average 269.5 ng m⁻² day⁻¹ (TGM = 24 ng m⁻³), 352.5 ng m⁻² day⁻¹ (TGM = 37 ng m⁻³), 1079.6 ng m⁻² day⁻¹ (TGM = 160 ng m⁻³) and 49.2 ng m⁻² day⁻¹ (TGM = 160 ng m⁻³) in total darkness.
- The absorption process includes factors such as stomatal conductance, TGM level and relative humidity as limiting influences, while the desorption process appears to be more complex and involves not only stomatal conductance and temperature, but also CO₂ concentration and Hg speciation in the leaf interior, amongst other factors. More research is needed in order to ascertain the role of relative humidity in the exchange process.
- The reversible/non-reversible nature of the Hg uptake process must be reconsidered bearing in mind that Hg in the leaf interior can be emitted if changes in the ambient conditions are sufficient to favour this process.
- The most relevant change in ambient conditions that can promote Hg emission from vegetation is a decline in TGM level. This could be relevant in the context of climate change and the reduction in anthropogenic Hg emissions, with a slowing in the decrease in TGM levels after the reclamation of polluted areas if vegetation increases its desorption rates as TGM declines.

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