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Fine-tuning the use of moss transplants to map pollution by Potentially Toxic Elements (PTEs) in urban areas

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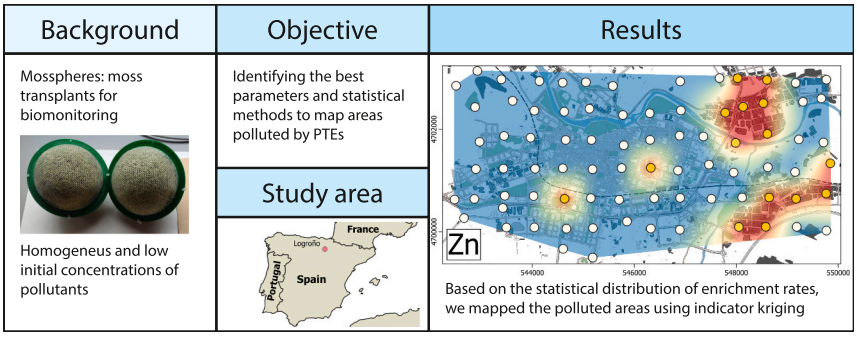
HIGHLIGHTS

- Mosspheres are optimized transplants of dried cultured moss for biomonitoring use.
- Monitoring polluting elements with mosspheres needs adapted statistical methods.
- The best index to express their pollutant concentrations is the enrichment rate.
- Indicator kriging can be used to accurately identify polluted areas.
- The sampling design should be carefully considered to detect point pollution sources.

GRAPHICAL ABSTRACT



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ABSTRACT

Mosspheres are a kind of moss transplants which offer a novel approach for detecting atmospheric pollution using devitalized mosses, as they reflect the atmospheric deposition of certain elements and polycyclic hydrocarbons. However, due to the unique features of the mosspheres such as the low elemental concentrations in the cultured material, the data treatment needs to be different from that of conventional biomonitoring studies. In this article, our objectives are to identify the best parameter for expressing the levels of chemical elements accumulated by mosspheres, and to apply a recently developed method to assess the probability of pollution of each sample and of the study area. To do this, we used data from a study in which 81 mosspheres were exposed in a medium-sized city in southwestern Europe.

Comparing different pollution indices, we selected the enrichment rate (ER) as the most useful, as it is resilient to fluctuations in the initial concentrations and takes into account the time factor, allowing for greater comparability among studies. Then, we determined that the statistical distribution of the ERs of most elements fitted a normal distribution, showing that most samples did not differ significantly from the background concentrations for these elements. On the other hand, for Ni, Pb and Zn there was a subpopulation of samples above background values. In these cases, we determined the probability of pollution of each sample. Finally, we used indicator kriging to calculate the probability of pollution across the study area, identifying the polluted areas,

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which for some elements match the distribution of the main industries and highways, indicating that this is a suitable protocol to map elemental pollution in urban areas.

1. Introduction

Atmospheric pollution by potentially toxic elements (PTEs) poses a significant risk to human health (Ali et al., 2019; Lelieveld et al., 2015), particularly in urban areas where it is most pronounced. In order to protect the population from this threat, detailed information on pollutant distribution is essential, as it enables the accurate identification of affected areas, facilitating the implementation of necessary interventions. The most direct approach to obtain this information is to use atmospheric deposition collectors or high-volume air samplers (Amodio et al., 2014; Hart et al., 1992), but the cost and complexity of this technique can limit their application in studying spatial distribution over large areas with sufficient resolution.

An alternative approach that has been used to address these shortcomings is biomonitoring: measuring the concentrations of pollutants in organisms rather than in the air or atmospheric deposition. Mosses, in particular, have been used for studying PTEs in the atmosphere due to their abundance, tolerance to stress, and widespread distribution. The simplest application the biomonitoring technique involves collecting mosses from their native habitats in the study area and determining their elemental concentrations, which provide valuable information about the abundance and bioavailability of the pollutants to which they are exposed (Harmens et al., 2010). However, since the inception of the technique in the late 60s (Rühling and Tyler, 1968, 1970), numerous advances have improved its reliability and applicability. In the 70s the collection of mosses to create transplants intended to be exposed in the study area (also known as the "moss bag technique", a type of active biomonitoring) was proposed (Goodman and Roberts, 1971; Little and Martin, 1974). This approach offers advantages such as the use of a more homogeneous material (Di Palma et al., 2016; Fernández et al., 2002), the flexibility to apply it in locations where the moss species used is not present, known exposure times for better interpretation, and optimization of the sampling design. This method also opened the possibility of pre-exposure treatments such as devitalization, which stops plant metabolism and growth (Fernández et al., 2009), ensuring more consistent results without impeding pollutant accumulation. Additionally, devitalization simplifies logistics, as dried transplants can be prepared and stored in the laboratory (Giordano et al., 2009). Finally, a last push towards higher reliability and homogeneity was the use of cultured material, guaranteeing low initial concentrations and availability on demand (Beike et al., 2015).

Unfortunately, the diversity of methodologies, combined with the use of different species and exposure protocols, hindered the widespread application of biomonitoring, as it limited the comparability among different studies. This challenge created a need for standardization that was addressed in a review by Ares et al. (2012), which recommended using transplants of mosses from the genus *Sphagnum*, cultured in the lab, and oven-dried before exposure. The culmination of this standardization effort was the European project MOSSClone (Beike et al., 2015). In this project, a *Sphagnum palustre* clone was cultivated, characterized from a physicochemical point of view (Di Palma et al., 2016; Gonzalez et al., 2016), and packaged in plastic spheres forming mosspheres, a version of the "moss bags" which ensures even and optimal exposure (Capozzi et al., 2016).

This project optimized the protocol for obtaining moss transplants which have been proved to capture high amounts of pollutants (Capozzi et al., 2017) and to reflect the atmospheric deposition of particles and some pollutants associated to this process (Aboal et al., 2020; Pacín et al., 2023). Nevertheless, the data obtained using mosspheres show some differences when compared with direct environmental measurements, passive biomonitoring, and even active biomonitoring with material collected from nature, making its interpretation less straightforward.

In passive biomonitoring studies, as in those making direct environmental measurements, pollution in the samples is often assessed using indices that compare sample concentrations with background values such as those on the earth crust or soil, but differences between both materials can render their interpretation unreliable (Kłos et al., 2011). Active biomonitoring solves this issue by providing a more direct reference for comparison: the pre-exposure concentrations of the material. This is frequently used in indices such as enrichment or accumulation factors (EFs) and the relative accumulation factor (RAF) (for examples see (Aničić et al., 2022; Arndt et al., 2017; Demková et al., 2017; Levei et al., 2020). However, whereas the initial concentrations of mosses collected from nature can be relatively high and variable, the concentrations in cultured mosses are minute (Di Palma et al., 2016), leading to different challenges. The use of transplants with collected material can lead to pre-exposure concentrations higher than those of some samples after exposure, hindering their application for slightly polluted areas. In these regions, differentiating between the variance caused by the initial concentrations and by the uptake during the exposure period can be so problematic that some authors developed techniques to assess the sensitivity of this type of biomonitoring (Couto et al., 2004). The low concentrations of pollutants of cultured mosses solve this problem, but the application of EFs and RAFs can still be problematic. As these indices are calculated by dividing by the initial concentrations of the material, any contamination in the unexposed samples can lead to a many-fold decrease in the final value. Additionally, if the initial concentrations fall below the limit of quantification of the analyzer, they cannot be calculated. Another set of indices that have been less popular are those calculating the difference between initial and final concentrations, which can then be normalized by the length of the exposure period (such as the relative accumulation rate [RAR] and enrichment rate [ER]) or not (such as the net enrichment [NE]) (for examples see (Aprile et al., 2010; Couto et al., 2004). These indices should be less sensitive to variations in the initial concentrations because even if the starting values have a high percentual change, if they are still much lower than the final concentrations, the index would be stable.

Another issue is recent studies suggest that the relationship between the concentrations in moss transplants and in the air they are exposed to is strong only for some pollutants such as heavy PAHs (Aboal et al., 2020; Pacín et al., 2023) and a few PTEs like Cu (Ares et al., 2015; Boquete et al., 2020), but for most elements the correlations are not significant. While the technique still has the potential to differentiate between polluted an unpolluted places qualitatively (Boquete et al., 2015), the specific values may not reflect those found in the air. To address this issue, Giráldez et al. (2022) proposed a novel method to identify polluted locations and to map the probability of contamination in the space between them using moss biomonitoring. However, this method has yet to be tried with mosspheres.

Thus, the objective of this study is to optimize the processing of elemental information from mosspheres in two ways. The first way is to compare the indices that could be used to represent the elemental pollution detected by mosspheres, aiming to select those allowing for greater robustness and comparability. The second approach is to use the selected index to establish which sites are polluted by each element. These aims were tested using the data from a study in which 81 mosspheres were exposed in a medium-sized city (around 150,000 inhabitants).

2. Material and methods

2.1. Mossphere preparation

The mossphere (Mosspheres®) is a biotechnological device developed within the FP7 European project "Monitoring Air Quality Using Moss" ("MOSSclone"), in which lab-grown *Sphagnum palustre* L. was used for atmospheric biomonitoring. The plants were grown in vitro under axenic conditions in 15 L photobioreactors (Applikon Biobundle) with liquid Knop medium (Reski and Abel, 1985) supplemented with micronutrients, sucrose and ammonium nitrate at pH 4.8 (for more information see Beike et al., 2015). After a 4-week growth period each bioreactor produced approximately 300 g d.w. of material, which was drained and washed with bidistilled water and 10 mM EDTA (1 L of solution per 200 g d.w.) to remove any residual PTEs, including the Cu and Zn provided as micronutrients. The moss material was devitalized by oven drying in gradual stages at 50, 80, and 100 °C, each of 8 h in duration (Ares et al., 2012; Debén et al., 2016). Under these conditions, the moss material was grown free of PTEs.

The mossphere consists of two concentric empty spheres, each formed by two hemispheres; the internal sphere made of pierced high-density polyethylene (Ø 100 mm), and the external sphere (Ø 110 mm), made of a 2 mm mesh nylon net. The space between the two spheres (10 mm thick) is filled with 3 g of the devitalized clone, obtaining a dry mass/surface ratio of the sphere of 10 mg cm^{-2} (Capozzi et al., 2016).

2.2. Sampling design

The study was carried out in Logroño (La Rioja, northern Spain), a city with 151,113 inhabitants (INE, 2018), and a 91,489 vehicle fleet (DGT, 2019), with a highway bypassing the main residential area through the East and South, and two industrial districts located at the Northeast and Southeast. A total of 84 mosspheres were exposed, but only 81 remained at the end of the exposure period. Out of these, 76 were distributed in a regular mesh of 575 m on each side, which covered the city and its immediate peri-urban area. In addition, 5 mosspheres were placed in sites outside of the limits of the city which a priori are not directly affected by urban pollution (Fig. 1).

At all points of the regular grid, the spheres were hung on fiberglass poles, which in turn were held perpendicularly over streetlamps. The additional spheres were placed on ad-hoc poles. In all cases they were at a height of 4 m. Prior to their placement in March of 2018, the mosspheres were stored in vacuum-sealed bags and only opened at the time of exposure. After an exposure period of approximately 3 months (Capozzi et al., 2016), mosspheres were removed from the poles and immediately stored in airtight plastic bags for transport to the laboratory. Five other mosspheres were kept hermetically sealed in their original bags and stored during the exposure period in order to be used as transportation controls. At the end of this period, the sealed bags were opened and the control mosspheres were thus taken to all collection points to check for contamination during transport. Once in the laboratory, the bags containing the exposed mosspheres were opened for drying at room temperature. The moss material was then extracted from each sphere for analysis. Three samples of the initial material were kept in the laboratory to be used as a pre-exposure reference (T0 controls). At the end of the exposure period, the 81 exposed samples, the 5 transportation controls, and the 3 T0 controls were analyzed following the same procedure.

2.3. Chemical analysis

The moss samples were dried in an oven (at 40 °C, 24 h) and homogenized in a ultracentrifugal mill with zirconium vessels and balls (Restch MM-400). Approximately 0.2 g of each sample were digested in Teflon bombs with 8 mL of HNO_3 (Hiperpur) and 2 mL of H_2O_2 (30 %) in a microwave oven (Ethos-1, Milestone) at high pressure, increasing the temperature to 190 °C for 25 min and maintaining this temperature for 15 min. After cooling, the extracts were transferred to volumetric flasks and diluted (to 25 mL) with MilliQ water. The concentrations of Ca, Cd,

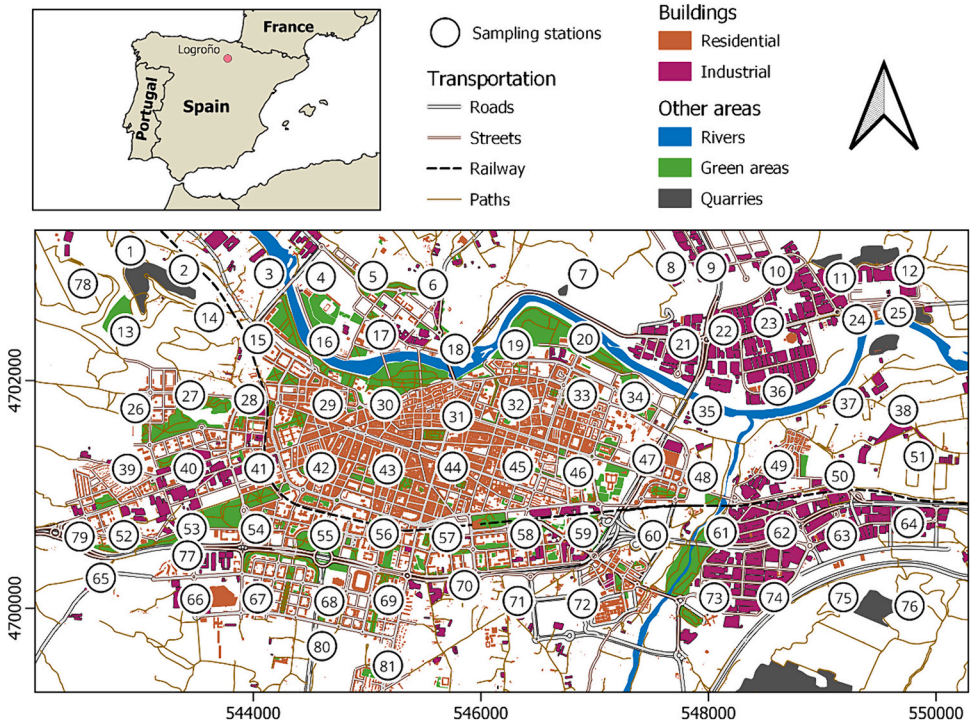

Fig. 1. Map of sampling sites in the city of Logroño, including the distribution of possible sources of pollution such as transportation networks and industries, as well as less polluted areas such as green spaces. Mosspheres 77 through 81 were placed in supposedly clean sites in the periphery of the city. The inset in the top left corner shows the location of the studied city in the Iberian Peninsula.

Cu, K, Mg, Mn, Na, Ni, Pb, Rh, Sb, V and Zn were determined by inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7700×) at the Research Support Services Unit (University of Santiago de Compostela). Determinations of As and Pd were conducted by the Research Group in Trace Elements, Spectroscopy and Speciation with the more sensitive high-performance liquid chromatography-inductively coupled plasma mass spectrometry (HPLC Flexar, ICP-MS NexION 300× PerkinElmer). In the case of Hg, an elemental analyzer (Milestone DMA 80) was used in which the homogenized dry material is introduced directly in the analyzer.

Standard reference material M3 (the moss *Pleurozium schreberi* (Brid.) Mitt.; (Steinnes et al., 1997) was analyzed for quality control once every 10 samples. To calculate the percentage of error in the analysis (Čeburnis and Steinnes, 2000), analytical replicates were analyzed also once every 10 samples. For most elements, the recovery percentage of the certified reference material was in the 100 ± 15 % range (K, Mn, Ni, Na, Pb, Zn) or 100 ± 25 % range (Ca, Cd, Cu, Hg, Mg, V), with <10 samples below the limit of quantification (LOQ), and a global error lower than 15 %. However, As and Rh had worse quality results. Both elements had a high degree of variability among replicates, 18 % and 28 % respectively, and the recovery of the reference material for As was low, at around 62 % on average. Thus, the results for these elements were kept in the study on the grounds that their values were coherent with the results for other elements, but the reliability of the measurement should be kept in mind.

2.4. Data treatment and pollution indices

We considered that the differences in the concentrations of the pre-exposure material that was kept in the laboratory and the transportation controls were due to contamination during transport. Thus, the difference between the average of these two groups was subtracted from the values of the samples, as to only preserve the uptake that happened during exposure.

We calculated the enrichment factor (EF) as described in the literature (e.g. Sandu et al., 2012), using Eq. (1).

$$EF = \frac{[X]_{after}}{[X]_{before}} \quad (1)$$

where $[X]_{before}$ and $[X]_{after}$ are the elemental concentrations before (T0 controls) and after the exposure period.

For the calculation of the enrichment rate, we used Eq. (2).

$$ER = \frac{[X]_{after} - [X]_{before}}{t} \quad (2)$$

where $[X]_{before}$ and $[X]_{after}$ are the same as before, and t is the duration of the exposure period in days. Further statistical analyses are made using ER rather than elemental concentrations.

The formula for net enrichment (NE) is not shown as this index is not used in our study, but is simply the difference between pre and post-exposure concentrations. The same is true for RAF, which is calculated as EF, but using NE rather than post-exposure concentrations in the numerator.

2.5. Statistical analysis

All analyses were performed using R software (R Core Team, 2022). To determine the sampling sites for which the concentrations of each element were higher than the background we followed the method used in (Giráldez et al., 2022). Briefly, we started by identifying the upper outliers in the distribution of each element using Tukey's method for outlier detection (Eq. (3)), which were considered as polluted.

$$Out > Q_3 + 1.5IQR \quad (3)$$

where *Out* are the outliers, Q_3 is the third quartile, and IQR is the interquartile range.

Then, we divided the elements in two groups, those that are better modelled by a Gaussian distribution, and those that better fit a mix of two normals. For the latter, one of the components contained the samples with background values (the one with lower average), and the other the ones affected by local pollution sources. The function *boot.comp* from the package mixtools was used to determine the best number of components to model the distribution of each element. This step was performed 10 times to ensure the robustness of the result. For the elements in which a mixture was the best model, the model parameters were estimated using an EM algorithm, using the functions *normalmixEM* (package mixtools) and *norMix* (package nor1mix). Then, the function *dgeometric.test* from the package GoFKernel was used to test the significance of the goodness of fit. We obtained the probability of each sample to belong to each component from the modelled distributions. Samples with a probability of belonging to the first component >0.5 were considered as part of the background component, and the rest as polluted.

Later we tested the distribution of polluted samples by each element for spatial structure using the function *sm.variogram* (package sm). In the samples for which spatial structure was detected, we applied indicator kriging models to map the probability of each area of being polluted, using the packages sm and gstat.

2.6. Visualization

All maps were created using QGIS 3.28.3. The information about the buildings, public infrastructure and land use of the city was obtained from the Spanish Centro Nacional de Información Geográfica (https://centrodedescargas.cnig.es/CentroDescargas/catalogo.do?Serie=CAANE), and the information about countries' boundaries was obtained from the website public.opendatasoft.com . The data overlayed on these maps is the probability of pollution of each sample for the elements without spatial structure, and the probability of pollution of the study area for the elements with spatial structure.

3. Results

The elemental concentrations in the mosspheres are shown in Table 1SM of the supplementary material. For all PTEs, the concentrations of both T0 controls and transportation controls were much lower than those of exposed samples. However, for some elements such as Cu, Hg, Pd and Pb there were notable differences between the concentrations in T0 controls and in transportation controls, the latter having higher concentrations, which indicates that some contamination occurred during transport to and from the study area.

3.1. Descriptive parameters

Table 1 shows the average ER and EF for each element, calculated using the average, maximum, and minimum values of the T0 controls. The last two columns represent the difference between using the minimum and maximum values of the controls, which for most elements was higher in the EF, with the only exceptions being Hg, K, Mg and Na. These elements either had extremely low concentrations (Hg) or are nutrients whose concentrations remained nearly constant or decreased during the exposure period (K, Mg and Na).

The last row shows that, on average, the variation on the initial values leads to a 20 % difference in the ERs, whereas the EFs changed 50 %. Based on this result, all calculations and maps in the next section were done using ER.

3.2. Spatial distribution of pollution

After removing outliers, only 3 of the 16 elements studied were

Table 1
Average of the enrichment rates (ER) and enrichment factors (EF) of all the samples for each element, using the mean, minimum, and maximum of the controls. The percentual differences in ER and EF between the values using the minimum and maximum of the pre-exposure (T0) controls are also shown. The units for the ER values are mg kg^{-1} day^{-1}. EF and the percentual differences are unitless.

	Mean controls		Min controls		Max controls		% dif max-min	
	ER	EF	ER	EF	ER	EF	ER	EF
As	0.00329	38.0	0.00329	38.0	0.00329	38.0	0	0
Ca	72.6	3.48	68.5	3.05	77.5	4.17	13.1	36.9
Cd	2.61E-4	4.10	2.60E-4	4.08	2.61E-4	4.11	0.215	0.667
Cu	0.0878	5.82	0.0855	5.17	0.0918	7.47	7.38	44.4
Hg	9.80E-5	1.77	7.03E-5	1.45	1.29E-4	2.32	83.1	60.1
K	−83.6	0.340	−99.4	0.303	−73.2	0.371	26.3	22.5
Mg	4.38	1.23	2.96	1.14	5.10	1.28	72.5	11.6
Mn	0.211	2.41	0.168	1.87	0.237	2.92	41.1	55.9
Ni	0.0218	5.74	0.0195	3.82	0.0228	7.32	16.9	91.5
Na	−17.9	0.381	−18.4	0.375	−17.2	0.391	6.70	4.37
Pd	9.23E-4	4.11	8.07E-4	2.95	0.00105	7.29	30.6	147
Pb	0.0329	42.9	0.0328	36.5	0.0331	57.2	1.02	56.8
Rh	3.11E-6	16.4	3.11E-6	16.3	3.11E-6	16.4	0.0433	0.667
Sb	0.00509	5.64	0.00462	3.97	0.00574	14.1	24.2	255
V	0.0191	138	0.0191	137	0.0191	138.19	0.00486	0.667
Zn	0.392	2.81	0.361	2.46	0.409	3.05	13.4	24.3
Average	−1.48	17.1	−2.85	16.2	−0.431	19.0	21.0	50.8

better explained by a mix of two normals rather than a simple normal distribution. Fig. 2 shows the distribution of the ER values for each element, the comparison with a normal distribution using the same average and dispersion parameters, and for Ni, Pb and Zn, the modelled distribution as a mix of normals.

For the elements modelled using two components, the likelihood of each sample belonging to each component was calculated, and the probability of belonging to the second component was considered the probability of that sample of being polluted. Outliers were directly assigned a probability of being polluted of 1. The results are displayed in Figs. 3, 4 and 5, and S1 of the supplementary material for all elements except nutrients (Ca, K, Mg, and Na), whose concentrations are controlled by factors other than anthropogenic pollution, and Mn, for which it has been proved that the moss bag technique is not effective (Boquete et al., 2011).

For the elements in which the distribution of polluted samples had a significant spatial structure, the likelihood of pollution of the study area for each element was calculated using indicator kriging. This technique uses binary data, so for the elements with two components, points were divided into polluted (including outliers and points with probability of belonging to the second component >0.5), and unpolluted (probability of belonging to the second component <0.5). The results for the affected elements are shown in Figs. 3 and 5.

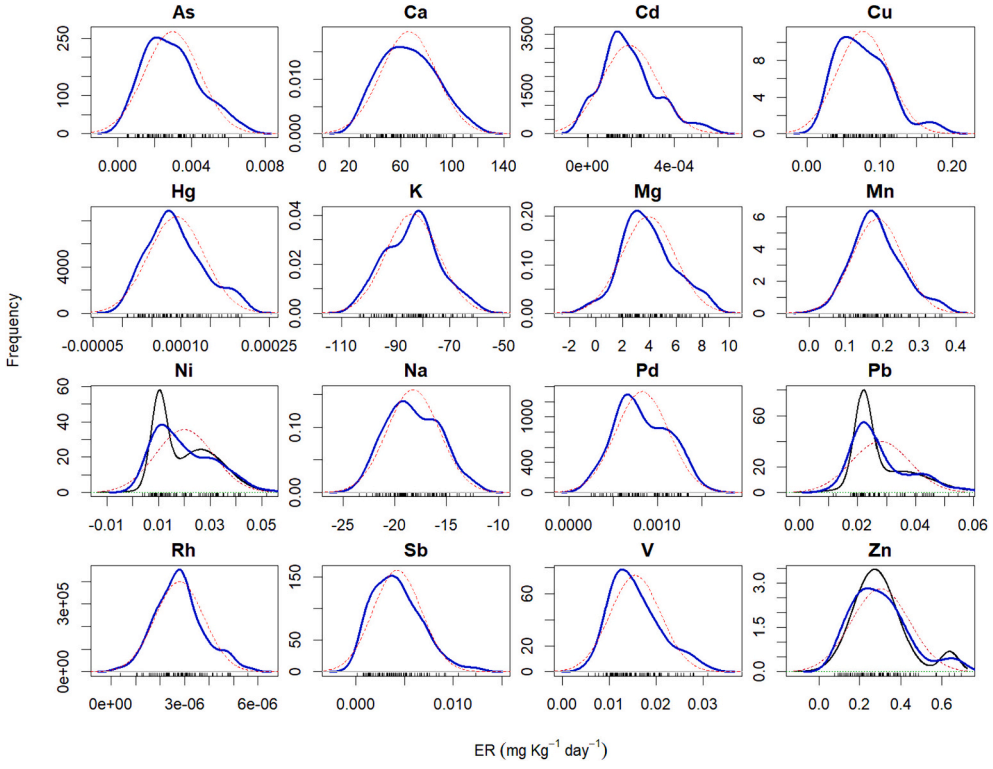

Fig. 2. Statistical distribution of the enrichment rates (ERs) of each element in the mossphere samples, after removing outliers. The blue lines represent a Kernel density estimation of the distribution of each element. The red lines are the modelled normal distributions. The black lines are the modelled mixes of normals.

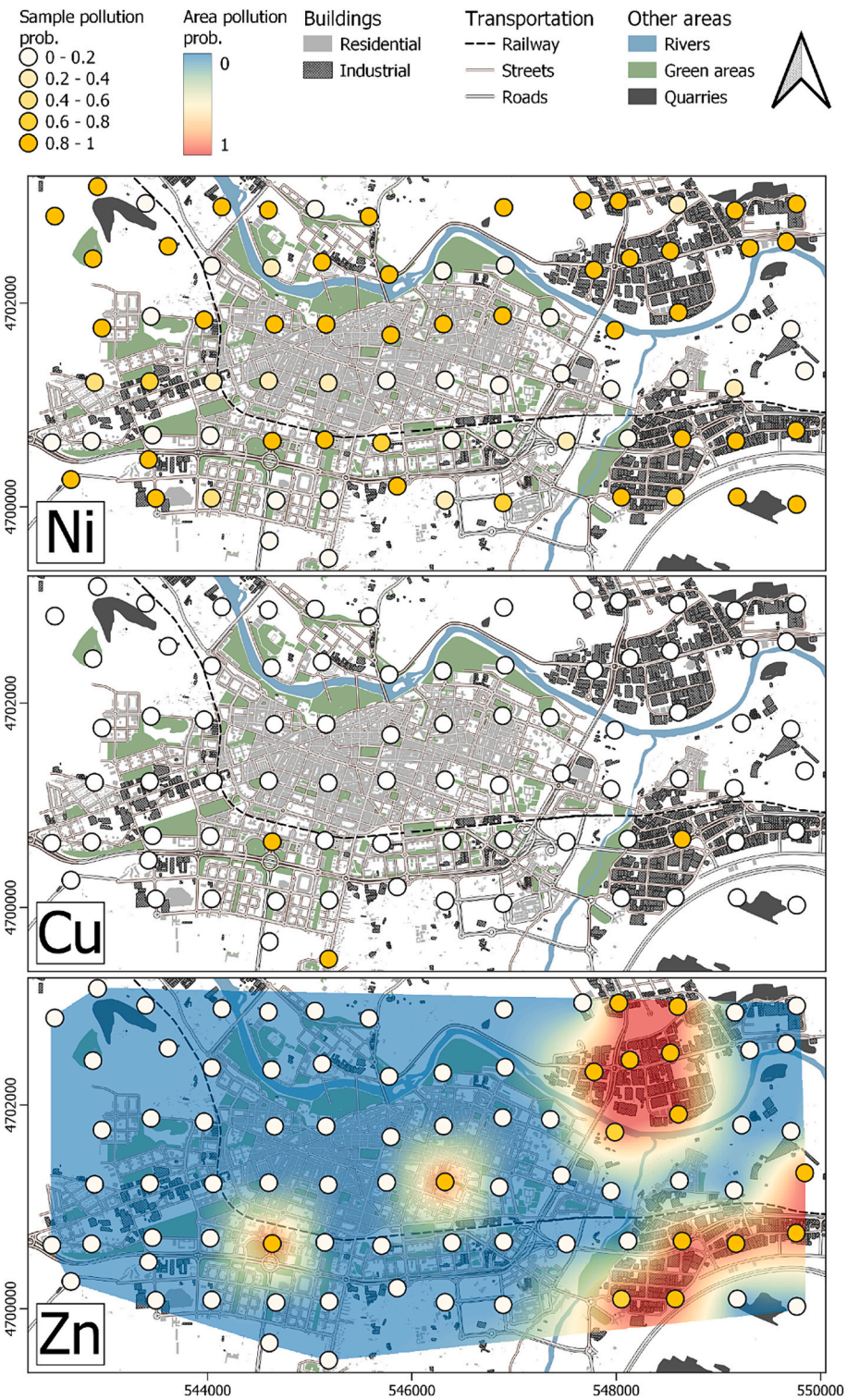

Fig. 3. Probability of pollution of each sample over the map of the study area for Ni, Cu and Zn. For Zn, the probability of each area of being polluted is also shown as a color gradient.

4. Discussion

4.1. Adapting the technique to low initial concentrations

Compared with another study that has used mosspheres to measure pollution in different European regions (Capozzi et al., 2016), the concentrations for most elements present in both studies (Cu, Hg, Ni and Zn) were very similar. The only exception to this was Pb, with average values significantly higher in Logroño.

Previous studies on active biomonitoring using moss collected from unpolluted areas faced the challenge of separating the contribution of PTEs from atmospheric deposition during the exposure period from the initial concentrations when these were high. This limitation led some authors to apply the calculation of the limit of quantification (LOQ) to the biomonitoring technique itself, to be able to tell apart these effects (Couto et al., 2004). The low concentrations found in the cultivated

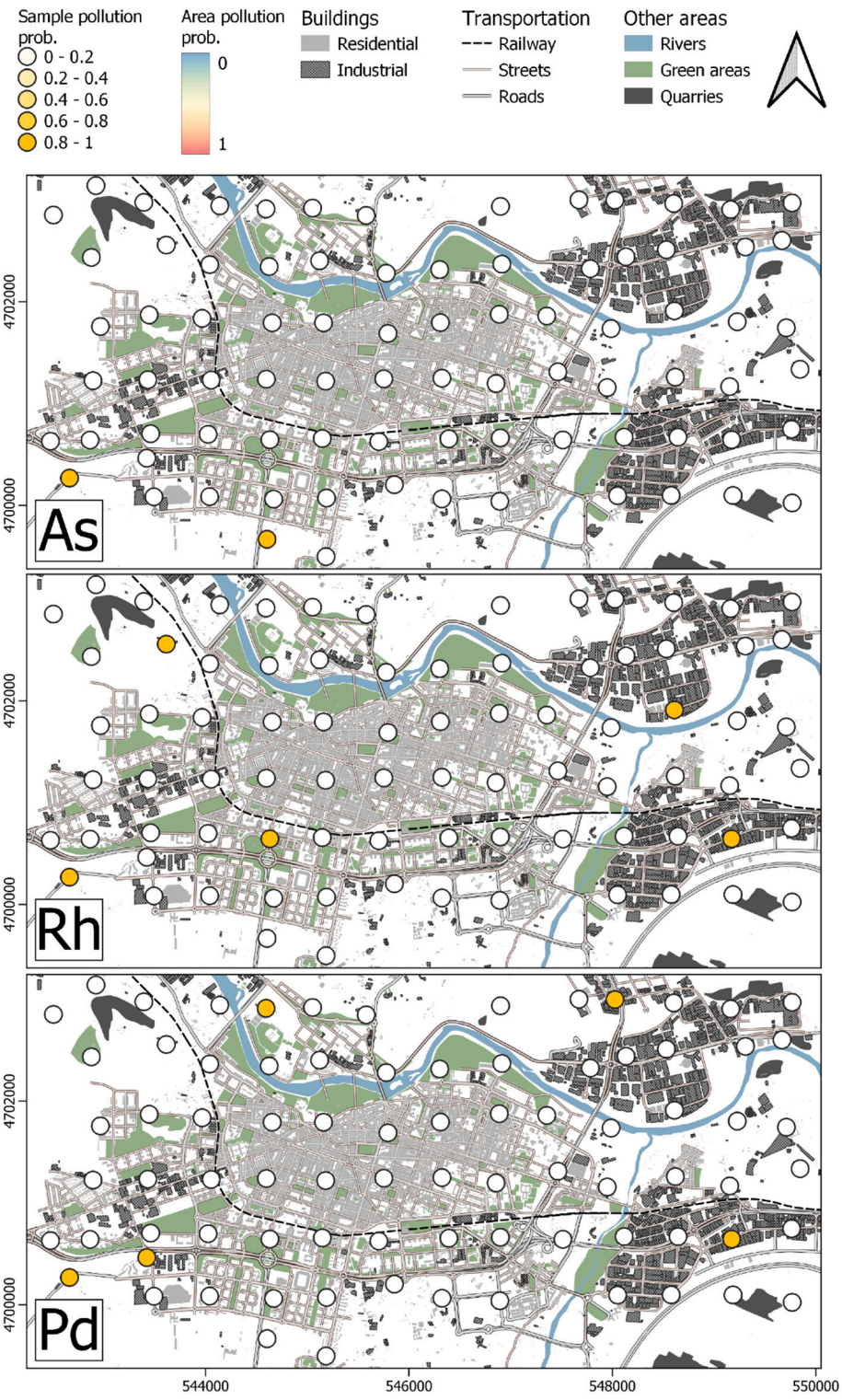

Fig. 4. Probability of pollution of each sample over the map of the study area for As, Rh and Pd.

moss used in mosspheres can solve this problem, but introduce new challenges, limiting the parameters that can be used to represent the data.

Several different indices and parameters have been used to express the pollution level of samples from active biomonitoring studies, but some of them have noticeable drawbacks. Indices that compare sample data with background environmental values, such as soil concentrations or geological elemental ratios, have been used in some cases (Aničić et al., 2009; Sergeeva et al., 2021), but the disparities between the reference and studied materials limit the precision in determining which samples should be considered as polluted. Most commonly, the indices used are factors such as the EF (Levei et al., 2020; Sergeeva et al., 2021) and RAF (Aničić et al., 2022; Sergeeva et al., 2021), which are calculated using the pre-exposure concentrations of the material in the denominator of the formula. This is a problem in the event of any contamination of the pre-exposure controls; if we take the concentrations of V in our experiment they went from around 0.01 mg kg^{-1} pre-exposure to around 1 mg kg^{-1} post-exposure. If there was contamination that raised

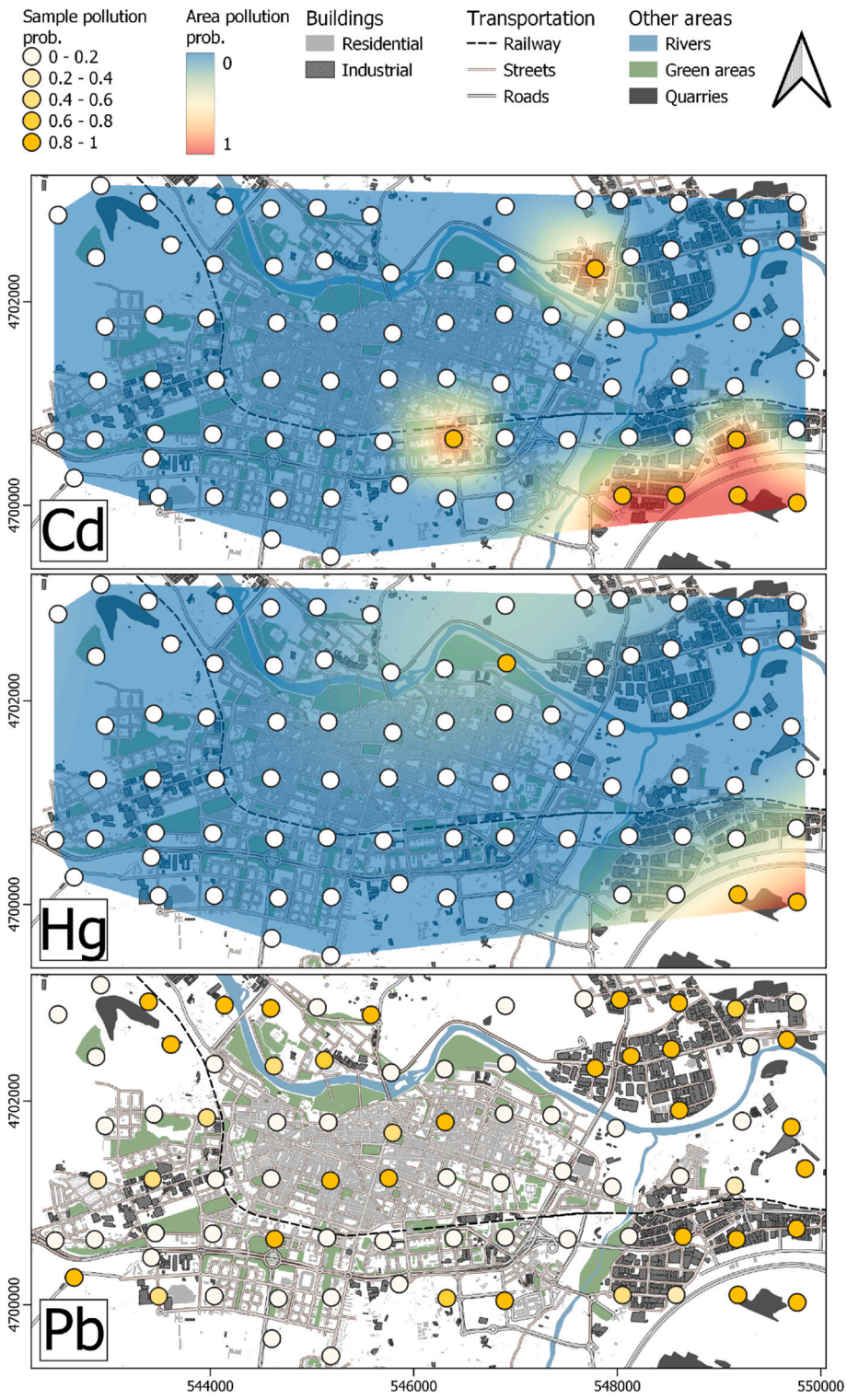

Fig. 5. Probability of pollution of samples over the map of the study area for Cd, Hg and Pb. For Cd and Hg, the probability of each area of being polluted is also shown as a color gradient.

the pre-exposure values to 0.1 mg kg^{-1}, which is still far from the concentrations after exposure, the EFs would decrease in one order or magnitude. Although the variance among the controls in our experiment is not so high, Table 1 shows that it is enough for this effect to be notable: the average difference between using the minimum and maximum values in our controls is 20 % for the ERs, but goes up to 50 % for EFs. In a scenario where the application of this technique is widespread and not every group using it grows the moss in-house, the initial variation could be much higher, as suggested by the increased concentrations in the transportation controls, which would make the use of EFs more unreliable.

In Table 1 we only compare EFs and ERs, but the percentage of variation would be identical between EFs and RAFs, and between ERs and NE, as these pairs of parameters have a 1:1 correlation.

Based on these findings, we recommend the use of enrichment rates (ERs) for biomonitoring studies using mosspheres. This choice has two

main advantages over other indices: 1) as aforementioned, ERs are more robust to variations in the initial concentrations; this is demonstrated in Table 1, which shows that for most elements, especially those with high pollution levels, ERs are less affected than EFs by the random variation of the pre-exposure concentrations; and 2) considering that the uptake rate is typically stable over time (Capozzi et al., 2016), dividing by the number of exposure days leads to greater comparability between studies with different exposure periods. The interpretation of this index is also quite intuitive, as it represents the uptake of PTEs per day of exposure.

4.2. Statistical distributions

The method developed by Giráldez et al. (2022) enabled us to accurately detect the elements for which a notable subpopulation of the samples collected in our study area had increased concentrations (Ni, Pb and Zn), and to assign a probability of being polluted to each sample. The remaining PTEs and nutrients better fitted a normal distribution. For PTEs, this indicates that there is not a population of points polluted by these elements, but only a few outliers. For nutrients (Ca, K, Mg, and Na), the mosspheres are not expected to be able to detect pollution for different reasons: the pre-exposure concentrations are already high (Di Palma et al., 2016); anthropogenic sources are not the main contributors of the environmental concentrations of these elements, and the devitalization process breaks the cellular membranes of the moss, allowing for the release of these elements (Capozzi et al., 2018). This explains the negative ER values for K and Na, which have high intracellular concentrations that decrease during the exposure period. Regarding Mn, despite not being a macronutrient like the rest, biomonitoring this element using mosses can be ineffective, which in previous studies has been shown to be controlled by other factors such as leaching from plants and competition with other elements that have higher affinity with moss (Boquete et al., 2011).

4.3. Spatial distribution of pollution

For most PTEs, the spatial distribution of polluted samples did not have a statistically significant structure, apart from Zn, Cd and Hg. However, this was consistent with the pollution sources of the city. The three elements that had significant spatial structure were associated with industrial areas, especially in the case of Zn, agreeing with previous studies that have shown that industry is a relevant source of these elements (Araújo et al., 2017; Pinot et al., 2000; Tchounwou et al., 2012). For Pd and Rh, elements usually associated with wear and tear of automobile catalytic converters (Kalavrouziotis and Koukoulakis, 2009), the points polluted by them are mostly distributed along the two main highways around the city.

Some sites displayed pollution by multiple elements, mainly those found in the industrial areas, but also some associated to high-traffic roads. This was the case for the sampling site 55, which was next to the highway exit leading to the city center, and was simultaneously polluted by Ni, Cu, Zn, Rh, Pb, and Sb, which are elements often connected with traffic pollution (Kalavrouziotis and Koukoulakis, 2009; Querol et al., 2007). These patterns were similar to those found for the concentrations of PAHs with 4, 5, 6 rings in these same samples (Pacín et al., 2023), indicating that these different pollutants have similar sources, mainly traffic and industry.

The reason why only the ERs of 3 out of 11 PTEs displayed spatial structure may be that the area of effect of the pollution sources is smaller than the grid of mosspheres, which could lead the concentrations to decay before reaching the next nearest one. If this is the case, only sufficiently extensive PTE sources, such as industrial districts, would produce data with spatial structure. Therefore, when designing the sampling strategy of an experiment aiming to obtain the spatial distribution of the probability of contamination, it is key to have enough resolution so that more than one sample is affected by point pollution sources, enabling the estimation of concentrations in between. Another shortcoming of this method is that, in some cases, the presence of a few polluted points in the corners of the sampling grid, can lead this test to detect a significant spatial structure, despite having only one or two polluted points. This can result in maps of pollution probability that show a gradient from the outside to the inside of the grid, as in the case of Hg. One way to address this issue would be to extend the sampling grid beyond the limits of the city, which was the initial intention for this experiment. However, there were logistical problems that made it impossible to hang the mosspheres in the streetlamp poles outside the city. Using a sampling grid dense enough to detect point pollution sources and sampling sites outside of the limits of the studied city, this method would ensure an accurate prediction of the distribution of PTEs in urban areas.

5. Conclusions

- The Enrichment Rate (ER) is a suitable index to represent the uptake of elements by mosspheres, due to its resilience to the variation of the initial values, and for taking into account the exposure period.
- The method developed by Giráldez et al. (2022) works both for determining which samples are polluted by each element and, in the cases where spatial structure is present, to map the distribution of the probability of pollution of the study area.
- The distribution of pollution by some PTEs matches the areas of the city affected by industry and transportation, further confirming the effectiveness of this technique.
- To achieve the best results applying this method, the sampling grid should have enough resolution so that every pollution source affects more than one mossphere, and should extend over the limits of the city to prevent artifacts caused by the edge of the grid.

CRediT authorship contribution statement

Antón Vázquez-Arias: Writing – original draft, Formal analysis. **Pablo Giráldez:** Software, Formal analysis. **Javier Martínez-Abaigar:** Writing – review & editing, Investigation, Funding acquisition, Conceptualization. **Encarnación Núñez-Olivera:** Writing – review & editing, Investigation, Funding acquisition, Conceptualization. **Jesús R. Aboal:** Writing – review & editing, Investigation, Funding acquisition, Conceptualization. **J. Ángel Fernández:** Writing – review & editing, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Javier Martinez-Abaigar, Jesus R. Aboal, J. Angel Fernandez has patent PASSIVE CONTAMINENT SAMPLING DEVICE pending to Albert-Ludwigs-Universität Freiburg. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Included in the Supplementary Material

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Declaration of generative Ai and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT to improve its langauge. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.171601.

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