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Lichens as bioindicators of atmospheric heavy metal deposition in Valencia, Spain.

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Abstract:

The lichens due to their symbiotic nature have unique characteristics that confer them a key role as bioindicators of the environmental contamination. Many investigations have been done using epiphytic lichens as bioindicators, but only a few of these studies have used epilithic-crustose lichens. Three different epilithic-crustose lichens species: *Candelariella* sp., *Lecanora* sp. and *Caloplaca* sp. were studied as bioindicators of V, Cr, Mn, Co, Ni, Cu, Zn, As, Rb, Sr, Mo, Cd, Sb, Ba, Pb, Bi and U trace elements. Inductively Coupled Plasma Mass Spectrometry routine procedure is used to determining these element concentrations. Two sites were selected for lichens sampling according to environmental contamination. The lichens were collected on the facade of the Santos Juanes church in an urban area of Valencia; and on the rural area of Albarracin. The main aim of this work is showing the efficacy of the epilithic-crustose lichens as bioindicators of the air pollution. This study shows that the city of Valencia, compared with the rural area has high levels of Cu and Pb as detected using lichens as bioindicators. Therefore on the basis of these results, it can be hypothesized that *Candelariella* sp., *Lecanora* sp. and *Caloplaca* sp. are good accumulators of air borne heavy metals.

Key words: Lichens; bioindicators; heavy metals; air pollution.

1 Introduction:

The large quantities of anthropogenic pollutants released into the atmosphere have a dramatic impact on air quality. Atmospheric pollution studies have therefore become an important area of research. The decline in air quality, due to the increase of atmospheric pollutants such as heavy metals, produces a variety of effects on living organisms and cultural heritage.
It is well known that heavy metals are present in the black crusts of historic buildings and sculptures in urban areas with high levels of air pollution. Black crusts are formed by the deposition of atmospheric particles and many studies have shown that a correlation exists between environmental pollution levels and crust formation and development (Ausset et al. 1996; Rodríguez-Navarro and Sebastian 1996; Grossi et al. 2003; Barca et al. 2010). These crusts cause significant aesthetic damage to cultural heritage, leading to the blackening of facades, ornamental details, and polychromes; substrates may also deteriorate due to the differences between the physicochemical properties of the black crusts and the original stone material. For example, differences in thermal behavior can lead to the fracturing of the crust and the consequent disintegration and loss of the original stone material (Winkler 1997; Warscheid and Braams 2000; ICOMOS-ISCS 2008; Dakal and Cameotra 2012).

An abundance of metals in the air can also have a negative impact on living organisms (even humans) due to their toxicity in high concentrations (Cicek et al. 2001). However some organisms, called bioindicators and biomonitors, are able to show these environmental pollutants providing useful information for the prevention and control of environmental pollution. Bioindicators are organisms that can be used for the identification and qualitative determination of the presence of air pollutants. Bioindicators respond to environmental pollution with changes in their vital functions or by pollutant accumulation in their tissue, thus providing information about the environment in which they are living (Tingey 1989; Ausset et al. 1996; Rodríguez-Navarro and Sebastian 1996; Grossi et al. 2003). However, biomonitors are organisms that can be use to provide quantitative information on the quality of the environment, and further for indicating long term trends including recovery (Hawksworth et al. 1989; Showman 1990). Lichens are also very good bioaccumulators of trace elements, since the concentrations found in their thalli can be directly correlated with those in the environment (Conti and Cecchetti 2001)

Lichens were first recognized as bioindicators in the 19th century. A great deal of research has been undertaken on the use of lichens (specially epiphytic lichens) as active (using transplantation) and passive (using lichen living “in situ”) bioindicators for the study of environmental pollution such as acid rain, heavy metals, oil spills, radionuclides air pollution and contamination from sulphur dioxide spills (Nylander et al. 1866; Ferry et al. 1973; Nimis et al. 1990; Bates et al. 1992; Richardson 1992; Stork and Sanways 1995; Nimis et al. 2002; Kircher and Daillant 2002). The extensive use of lichens as bioindicators of environmental pollution is due to their intrinsic characteristics which make them ideal for this purpose. Lichens have a high sensitivity to air pollutants, a widespread distribution, low mobility, are perennial and have a long life cycle (making long-term studies possible). Furthermore, they absorb air pollutants such as heavy metals because they take their nutrients primarily from the atmosphere (Purvis 1996; Gabriele and Callegaretti 2005). Some of the absorbed contaminants remain in the thallus cells via ion exchange mechanisms, and thus the pollutants accumulate inside the lichen (Brown et al. 1983). Detection of elevated levels of air pollutants inside lichens can therefore be correlated with the habitual presence of pollutants in the environment where they are located (Hawksworth 1990). A remarkable study was undertaken on Plimier Island Highway, United States in 1993. This study showed how levels of lead in lichens situated close to the highway increased significantly after the highway’s construction and significantly decreased after the use of the unleaded fuel became widespread (Lawrey 1993). When the air pollution is extremely high the lichens disappear for example Bergagli-Petricci (Bargagli-Pertrucci 1915) reported the absence of lichens in a 5km radius around a geothermic area in Italy due to high levels of air pollution. However, an interesting study shows that when the air quality of a region recovers, lichens quickly recolonize it. This study was developed in London in 1983 when a city centre thermal power station stopped working. The city centre was then recolonized by lichen species previously only found in the periphery (Hawksworth and MCManus 1989).

Numerous studies have been conducted using lichens as indicators of environmental pollution in different geographic areas. These studies have clearly demonstrated the great ability of lichens to accumulate heavy metals and their consequent potential as environmental pollution indicators. In 1989, a study revealed the presence of lead and cadmium contamination in the industrial area of La Spezia, Italy, using epiphytic foliose lichens (Benco et al. 1989). In 2001, Mangiafico and Pitruzzello used epiphytic foliose lichens to undertake an air quality assessment in the Melilli region of Italy, finding high levels of contamination of lead, copper and nickel (Mangiafico and Pitruzzello 2002). In 2005, Bretschneider and Marcano used epiphytic foliose lichens as indicators of heavy metal pollution in the Valle de Merida, Venezuela (Bretschneider and Marcano 1995). In 2010 another study conducted in Argentina using epiphytic lichen *Parmelia caperata* showed higher levels of iron, Manganese and Zinc in the city centre than in the control areas (Ghirardi et al. 2010). However, few studies have used epilithic-crustose lichens as bioindicators. Seaward studing *Lecanora muralis* epilithic-crustose lichen performance in urban environments couldn’t found Lead pollution zonation patterns (Seaward, 1976). In Israel, Garty et al. (1986 and 1977) achieved adequate air pollution study using the epilithic lichen *Caloplaca aurantica* showing higher heavy metal pollution in urban areas compared to rural control areas. Bajpai et al. (2010) undertook a biomonitoring study in India, employing *Lapraria lobifican* crustose lichens which demonstrated greater Fe accumulation than other species of folious and fruticulouse lichens (Bajpai et al. 2010). Epilithic crustose lichens have been also used in Brasil to biomonitor Arsenic in the environment (Deschamps and Matschullat 2011).

The authors’ preferential use of epiphytic lichens compared to epilithic lichens attends to different aspects like: the easier sampling (epiphytic lichens are easier to separate from substrate than crustose epilithic lichens); the easier handling of epiphytic lichens mainly when used on active transplant studies; and to the fact that epilithic lichens are generally slow-growing species. Some authors’ maintain that slow-growing species would be unreliable as their analysis would result of intermixing annually accumulated pollution contents (Hale and Lawrey 1985). But other authors defend that epilithic lichens slow down growth rate is an advantage because increases their life spam under the impact of air pollution (Temina 1998). It would seem, therefore, that further research is needed regarding the use of epilithic-crustose lichens as bioindicators.

The main aim of this paper is to assess the efficacy of using epilithic-crustose lichens as bioindicators of the air pollution of the city of Valencia, Spain. In addition, we compared the air quality of the city of Valencia with a less polluted rural area. The samples of the urban area were taken from the Santos Juanes church in Valencia (**Fig. 1**) situated in the city centre were traffic is dense almost 24 hours a day. Lichens of the same species growing in a rural area of Albarracín were studied. The rural area is located far away from cities and traffic contamination sources. Trace element concentrations, with particular attention to heavy metal content were determined. Three lichen species growing on the façade of the Santos Juanes church in Valencia (**Fig. 1**), and on a rural area of Albarracín were studied.

Moreover, this study can be important to investigate the relationship between heavy metal air pollution and diseases increase in the city of Valencia as other works have state. Cislaghi and Nimis (1997) established a correlation between contamination of heavy metals and frequency of lung cancer in Italian areas, using lichens to determine it. In 2001, Riccardi et al. conducted a study with epiphytic lichens in an area of Naples, Italy, succeeding in being able to relate high cases of digestive tumors with high levels of heavy metal contamination (Riccardi et al. 2001). Another Italian study demonstrated that hight concentrations of Pb detected in lichen thalli adjacent to a waste incinerator and a lead factory was related to relative high levels of Pb in local polulation blood tests (Palmieri et al. 1997).

2 Materials and Methods

2.1. The Santos Juanes church

The Santos Juanes is a Baroque church that was converted in 1240 from a Muslim mosque. It was declared 'Historic-Artistic National Monument' in 1942 (see **Fig. 1**). It is located in the old city center of Valencia and it is one of the oldest and most significant buildings of the city. The church houses the largest Valencian wall paintings, covering the vault and lunettes for a total of approximately 1200m^2. They were completed between 1693 and 1702 by artists Antonio Palomino and Guilló using the fresco technique where the pigments are applied with water and fixed to the mortars by carbonation processes.
Different species of lichens have grown on the upper part of the church facades and on the ceiling. Dense traffic occurs around the church every day, been the daily traffic density average between 1,908 and 11,397 vehicle per day in 2012 (Valencia's city council traffic information 2012).

2.2. Sampling

The sampling point selection was carried out according to the most abundant lichen species, present on the church facade and according to the different types of substrates where the lichens were growing: Godella limestone (**Fig. 2**) and brick.
To perform a comparative level of the air pollution of the city, we also collected lichen samples in the *Sierra de Albarracín*, Teruel growing on limestone. This control rural area is located 40km away from an urban area and therefore the contamination is minimal but the rain and sun exposure is similar. The lichen samples taken in the rural area were used as reference for good air quality.
The substrates in which lichens grow were also sampled and analyzed with the aim of separating in the lichens the elements taken from the air by those taken from the substrate.
A total of eleven samples including both epilithic-crustose lichens and substrates were taken by scalpel from each site, with an elevation of the ground level of around 10m in the control area and about 20m in the urban area (**Table 1**). Due to the fact that the lichens are in a historical building we took the minimum number of samples needed to do our studies. Each lichen sample was then divided into similar pieces that were used as a replicates. Each lichen sample piece was then separated into two parts. The first part was used for lichen identification and the second part was used to determine total lichen trace element concentrations.

2.3. Lichens identification

Each lichen sample was sectioned in two parts. One part was included in polyester resin and a thin section was prepared for lichen morphological identification by stereomicroscopic analysis, visible light, and ultraviolet light microscopic analysis (Zeiss Axioplan). The other one was analyzed by scanning electron microscopy coupled with energy-dispersive X-ray spectrometry (SEM-EDX Stereo-scan 440 LEICA Cambridge INCA Oxford EDS spot system) and by chemical test. For the SEM-EDX analysis, each lichen sample was placed on aluminum base adhered with conductive glue and after gold metalized**.** Chemical tests are based on the reaction between lichen substances and added reagents (KOH and sodium hypochlorite). The reactions produce color changes that give us information about the type of lichen substances that help us with the lichen characterization (Rodríguez-Navarro and Sebastian 1996). These optical and chemical analyses together with taxonomic tables let us recognize the most abundant lichen species found on the church.

2.4. Substrate characterization

The substrate samples were divided in two portions. One part was included in epox resin to prepare the thin sections (thickness of 30 micron) for microscopic study. The microscopic techniques were polarizing optical microscopy and scanning electron microscopy coupled with energy-dispersive X-ray spectrometry

(SEM-EDS) (SEM-EDX Stereo-scan 440 LEICA Cambridge INCA Oxford EDS spot system). Microscopic observations were carried out in order to define the petrographic features and to determine the major element concentrations of the substrates. The other portion was powdered to determine the trace element concentrations of substrates.

The characterization of substrate was carried out with the aim to determine the contribution of the substrate in the chemical composition of the lichens.

2.5. Trace element determination for substrates and lichens

Total trace element contents of lichens (on-tallus and in-tallus pollutant content) and substrates were determined using a quadrupole Inductively Coupled Plasma Mass Spectrometry (ICP-MS) Perkin Elmer, model Elan DRCe, by means of solution nebulisation. The ICP-MS is a fast, multielemental technique which offers the possibilty to analyse, with high precision and accuracy, a great number of trace elements. The other advantages of this technique are the use of limited quantities of sample (about 100mg of powder) and the very low detection limit (ppb). Because the epilithic-crustose lichens are strongly attached to the substrate, to successfully separate the lichen from the substrate without contamination, we cut with a scalpel only the superficial portion of each sample. Once the lichen thallus is separated from the substrate the trace elements can be determined avoiding substrate contamination.

The solution of the samples was obtained dissolving powders by acid digestion using the Mars 5 microwave apparatus (CEM technologies). In particular each lichen sample was milled in an agate mortar and successively 100 mg of powder were dissolved using 10 ml of HNO_3 of Merck ‘‘suprapure’’ grade within TFM digestion vessels. Similarly for each substrate sample 100 mg of rock powder were dissolved using a mixture of 6 ml HF, 4 ml HNO_3 and 3 ml $HClO_4$, within TFM digestion vessels; all reagents were Merck ‘‘suprapure’’ grade. The same procedure was used to prepare two standard reference materials [Argillaceous Limestone (SRM1d) from NIST and Micaschist (SDC-1) from the US Geological Survey]. These were used as quality control standards as suggested by Eggins et al. (1997).

After the digestion process all samples were diluted to 100 ml with Millipore^{TM} water and analysed by ICP-MS. The external calibration curves were prepared using the Merck “ICP multielement standard solution VI”. Internal standards (indium, germanium and rhenium) were added to standards and solutions.

The elements analysed were: vanadium (V), chromium (Cr), manganese (Mn), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), arsenic (As), rubidium (Rb), strontium (Sr), molybdenum (Mo), cadmium (Cd), antimony (Sb), barium (Ba), lead (Pb), bismuth (Bi) and uranium (U).

During the analytical sequence, the eleven specimens have been analyzed plus six quality control standards (three times Limestone and three times Micaschist) and five blank determinations, interspersed during the analytical run. To evaluate accuracy, the mean values of measurements carried out on the quality control standards were compared with those certified. Accuracies were better than 8%, with most elements within ±5%. The instrument detection limit was evaluated by multiplying the standard deviation of the blanks by 3.

2.6. Statistical analysis

ICP-MS data were submitted to analyses of variance (ANOVA) to evaluate statistical significant differences between the rural site and the urban site. The results of are shown by P values < 0.05.

3 Results and Discussion:

3.1. Lichen species found and identified

According to literature, lichen identification has been done by observation of morphological characteristics such as the growth form (biotype), the reproductive forms, the type and dimension of the spores (**Fig. 3**), the lichen color; and the color variation with chemical tests (**Fig. 4**). According to these lichen characteristics dichotomous keys have allowed us lichen identification (Lorente and Sanz 2000; Miani et al. 2006). The most abundant live lichen species present on the church were three. They were identified as: *Candelariella* sp., *Lecanora* sp. and *Caloplaca* sp. Ultraviolet light microscopy allow us to identify the alive lichens (**Fig. 5**)

All lichens found on the church are epilithic-crustose, so the absence of foliose and fruticose lichens gives us the first indications that the level of pollution of Valencia’s city centre is high (Quijada 2006).

Each lichen species have a particular pollutant concentration tolerance. If that tolerance is exceeded, lichen may die, therefore when air pollution is very high only the most resistant species will survive. The lichen growth form affects their tolerance and their airborne pollutants accumulation (Garty 2001). Epiphytic foliose and fruticose lichens are less tolerant to air contamination and therefore live in pure air areas; however epilithic-crustose lichens are more resistant to pollution, in fact they are able to live in more contaminated areas (Hawksworth et al. 2005). Bajpai (2009) demonstrated that foliose forms accumulate higher quantities of metals than crustose and squamulose lichens.

3.2. Substrate characterization

Petrographic observations revealed that the Godella limestone is a micritic limestone containing rare inclusions of clay. Allochemical components consist of various small bioclasts (algae and gastropods) of lake environment. Porosity is low (about 5%) and often the pores are filled by secondary calcite. Few grains of quartz and manganese oxides are also recognized.
The SEM–EDS results from Godella limestone showed CaO (around 98 wt %) as the major component, with very little amounts of SiO_2, Na_2O and MgO. Our analysis also showed that the physical and chemical properties of the Godella limestone and the "Sierra de Albarracín" limestone are similar. The second substrate was a brick characterized by a typical reddish color. The petrographic analysis allowed us to determine the relative amount of the matrix and clasts, and to distinguish between minerals and rock fragments. The matrix is well oxidized, brown-red in colour. The porosity is medium; often filled by secondary calcite. The abundance and grain size of mineral and rock fragments is variable (up to 1.5 mm of diameter). Spot analyses carried out by SEM-EDS on the matrix portion showed that it is characterized by the presence of SiO_2 and Al_2O_3 and subordinately by CaO, K_2O, MgO and FeO.

3.3. Trace element concentrations of lichens and substrates

The results of ICP-MS analyses of lichen and substrate samples, in ppm, are listed in **Table 2**. To evaluate the environmental pollution in the air of the Valencia city centre, the element increase has been calculated comparing the trace element concentrations in the lichens of Valencia with the concentrations in the lichens from the not polluted area (**Table 3**). The difference of element concentrations was calculated considering the type of the substrate (limestone or brick) where the lichens were found. This consideration was done because although the greater part of the heavy metals accumulated in lichen thallus are of atmospheric origin, several works have demonstrate a potential contribution of the substrate to the heavy metal content on the epilithic lichen thallus, even though it's contribution has a minor role (Garty et al. 1986).

From the study of trace elements content of limestone and brick present on the church facade (**Table 2**), high levels of V, Mn, Zn, Rb, Sr Cr and Ba can be seen in limestone and high levels of V and Sr in brick substrates (**Fig. 6**).

Using lichens as indicators, it is shown that there are higher levels of most of the measured heavy metals in the air of Valencia than the levels found on the rural control area. Only four elements show an important decrease compared with the control: Mn (-224.2 in *Candelariella* sp. on limestone; -144.7 in *Candelariella* sp. on brick; and -49.0 in *Lecanora* sp.on limestone), Ni (-29.2 in *Caloplaca* sp. in limestone), Zn (-24.4 in *Candelariella* sp. on limestone) and Ba (-16.2 in *Candelariella* sp. on limestone, and -15.2 in *Lecanora* sp. in limestone). This element decrease can be explained by large accumulation of divalent cation competitor (like Cu) abundant in the city of Valencia and not in the control area.

The most abundant elements found on all of the studied lichens with an increase of at least 20 ppm from the control lichens were: Cu, Sr and Pb in *Candelariella* sp. on limestone; V, Cr, Cu, Zn, Ba and Pb in *Candelariella* sp. on brick; Cu, Sr and Pb in *Lecanora* sp. on limestone; Cr, Cu, Zn, Ba, and Pb in *Lecanora* sp. on brick; V, Mn, Cu, Zn, Sr, Ba and Pb in *Caloplaca* sp. on limestone; and V, Mn, Cu, Zn, Rb, Sr, Ba and Pb in *Caloplaca* sp. on brick (**Fig. 7**). The differences found between element content on the same lichen species on the urban area, can be attributed to the different growing substrate (limestone or brick).

Statistical comparison of the element concentration (ppm) present in the lichens from the church site and from the rural site have shown significant differences ($p< 0.05$) on Cu, Pb, Co, Mo, Sb, and Bi.

The common elements found highly increased in all studied lichen species and not on their growing substrates were: Cu and Pb both of them showing statistically significant differences with the rural area ($p< 0.05$). The high levels of this two metals and its absence in the substrates proves that the reason for their accumulation is attributable to air pollution.

The research group of Neiboer descrived in 1978 that elemental uptake of lichens varied according to the elemental characteristic of the substrate, and of course to the environmental element composition

(Neiboer et al. 1978). Therefore the abundance of Sr found on the three studied lichens can be directly related to the high abundance of Sr in the substrates: limestone (517 ppm) and brick (533 ppm). The high increase of Zn, V, Rb, Mn and Ba found in all the studied lichens found on brick can be also associated to the high levels found of these elements in the brick substrate (see **Table 3** and **Fig. 7**). These abundant elements can be components or impurities of the church limestone and brick, or part of the atmospheric pollutants deposited on the church facade as black crusts. Our results are in accord with a recent study of Barca et al. (2011). They observed high levels of Cu, As, Co, Cr, Fe, Mn, Ni, Pb, Sb, Sn, Ti, V and Zn on black crusts present on buildings of the Catania city centre in Italy. This study demonstrates that these elements have part of its origin on anthropogenic sources like vehicular traffic and domestic heating.

According to the lichen-air model of Kularatne and de Freitas (2013) we can say that the metal content of this “in-situ” lichens are in equilibrium with the mean level of pollution in the valencia’s ambient air as they have been exposed to the environment for a considerable period of time. Following Rossbach et al. (1999) demonstrations about linear correlation between the element concentration in lichens and the distance from the emission sources. We can directly relate the high levels of Cu and Pb found on the church lichens with high city air pollution, and therefore assume that Valencia’s city air is polluted with Cu and Pb heavy metals. It is known that agricultural industries (fungicides and insecticides), pigments, construction, carbon and car combustion emit Cu and Pb into the atmosphere. Valencia is surrounded by important agricultural, ceramic and construction industries and the church has abundant traffic around it, therefore the air of Valencia has high levels of these heavy metals and for that reason the lichens accumulate them on their tissues. Our results agree with different studies carried out using epiphytic lichens as a biomonitors/bioindicators in Europe showing significant higher levels of Pb in urban areas compared to control areas even though the introduction of lead-free petrol, (Cardarelli et al. 1993; Deruelle et al. 1996; Monaci et al. 1997). In Italian cities high concentrations of Cu (Mangiafico and Pitruzzello 2002) and significant high levels of Cr, Cu and Pb (Loppi et al. 1998) were found. In Venezuela high concentrations of Pb were also found (Gordon et al. 1996); likewise, another study conducted on urban areas of Arizona, USA, found high concentrations of Zn, Cu, Pb, and Cd in lichens (Zschau et al. 2003). All these studies using lichens point to the vehicle traffic as the main source of atmospheric contaminants.

Our results are also in accord with studies performed using epilithic crustose lichen. A comparative study performed between urban, rural and suburban areas of Israel using *Caloplaca aurantia* showed generally higher amounts of Mn, Zn, Fe, Pb, Ni, Cu and Cr on lichen growing in ‘town’ than in ‘village’ areas (Garty et al. 1977). Our results also agree with another comparative study done in urban versus “health areas” in Germany using *Lecanora muralis* crustose lichen, where increased concentrations of Zn, Ag, Cd, Sn, Tl and Pb occur in large cities (Rossbach and Lambrecht 2006).

4 CONCLUSIONS

The study done on this work, using lichens as heavy metal content indicators shows that the city of Valencia has on its air high levels of Cu, Pb, showing significant differences ($p< 0.05$) when compared with the control site. Moreover, this study shows the suitability of *Candelariella* sp., *Lecanora* sp. and *Caloplaca* sp. epilithic-crustose lichens as bioindicators of air pollution.

The use of lichens as monitoring bioindicators gives important information because high concentrations of heavy metals in the air can be dangerous to human and Historic-artistic buildings health.

Moreover the two abundant heavy metals, Pb and Cu, found in this study in Valencia’s atmosphere can be toxic to human health. On the one hand, lead is very dangerous for the environment due to its high toxicity and because it cannot be naturally degraded chemically neither biologically. It’s accumulation on live organisms can severely affect them. Lead is a known neurotoxic and young children and fetus are at particular risk for exposure (ATSDR 2001). Lead poisoning in humans can produce health problems that go from headaches and insomnia, to coma and death. Lead atmospheric concentration is normally very low but lead mine extraction, refining, its industrial use, petrol combustion, smelters, batteries, pesticides and paints, may contributed to its high increment in urban areas (World Health Organization 2001). Due to the lead high damaging for human health, it remains one of the most epidemiological researched topics. From the other hand, high copper levels can damage the live organisms and producing kidneys problems and even cause cancer and death (Leone et al. 2006). Its air increment can be related to waste incineration, extraction, smelting, refining for its industrial use, and petrol combustion due to high traffic and heating plants.

This study provides baseline data on metal air concentration and its time accumulation at the city of Valencia. This information could be helpful for carrying out future monitoring and epidemiological studies in the area.

The exposed reasons on this study explain the importance of the study of air quality in urban areas both to control the "health" of the buildings and the health of the people who inhabit the area. Our work goal is that lichen can be valid, accurate, easy and economical environmental monitoring instruments, allowing also the comprehension of the evolution of air quality over time.

Indication of figures and tables

Fig. 1 Ancient map of Valencia's city centre. The Santos Juanes church of Valencia is marked with a white circle. Map by Tosca 1704, obtained from the Historical Archive of the City of Valencia.
Fig. 2 Sampling of *Candelariella* sp. from limestone substrate with scalpel.
Fig. 3 Electron microscopy image of a lichen spore.
Fig. 4 Light microscopy image of a positive, in red, chemical test lichen.
Fig. 5 Ultraviolet light microscopy image of *Candelariella* sp. lichen.
Fig. 6 Histogram showing the heavy metal content (ppm) on the two substrates of the church facade in which lichens grow.
Fig. 7 Histogram showing the element content increase on the three lichen species found on the church facade compared with the control lichens.
Table1 Sampling list describing: lichen species, substrate and sampling site.
Table2 Samples concentration values expressed in ppm of the seventeen elements measured by ICP-MS.
Table3 Element content increase of the sampled lichens in the church, regarding to the control lichen sampled on a non-polluted area.

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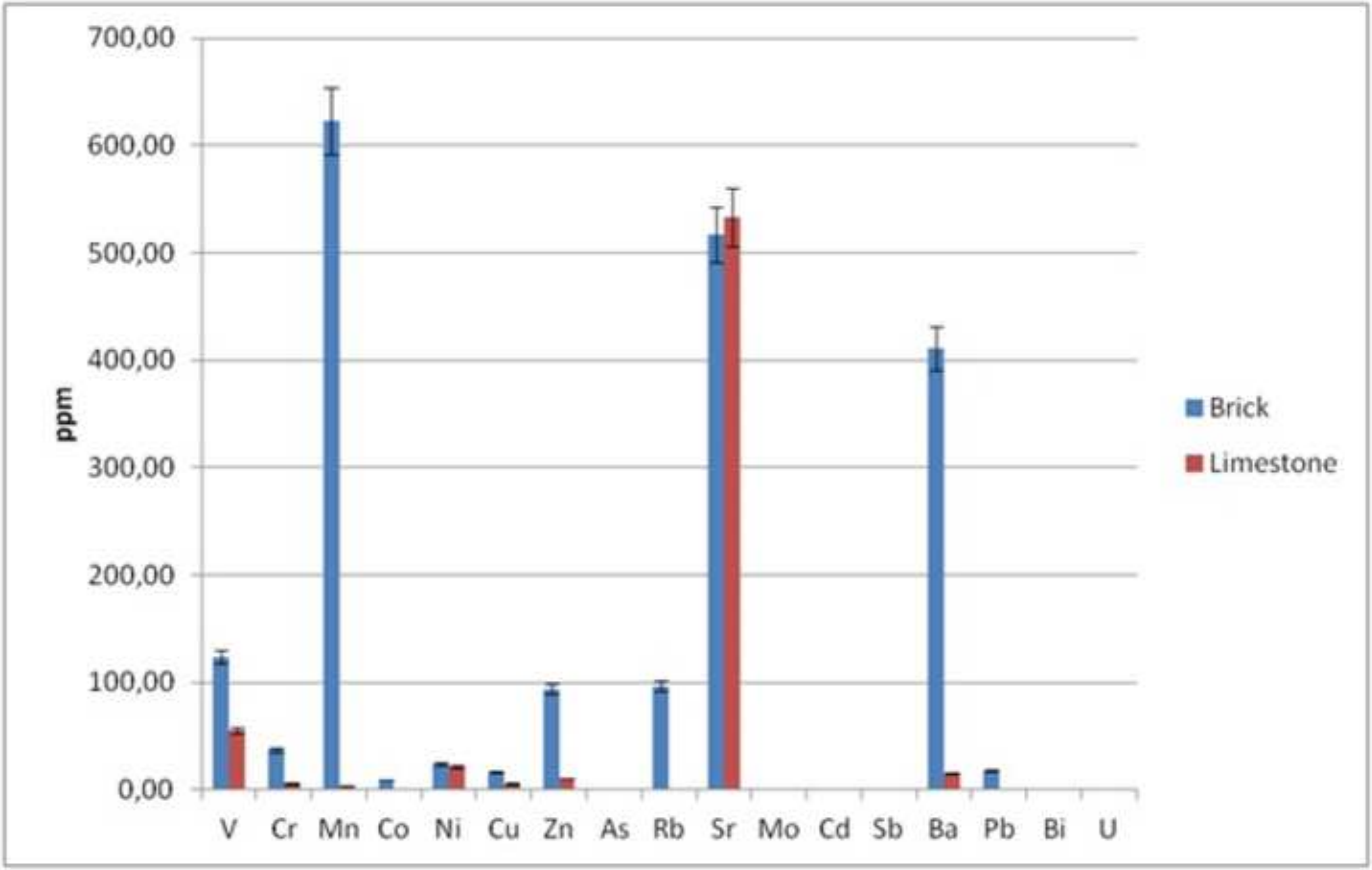
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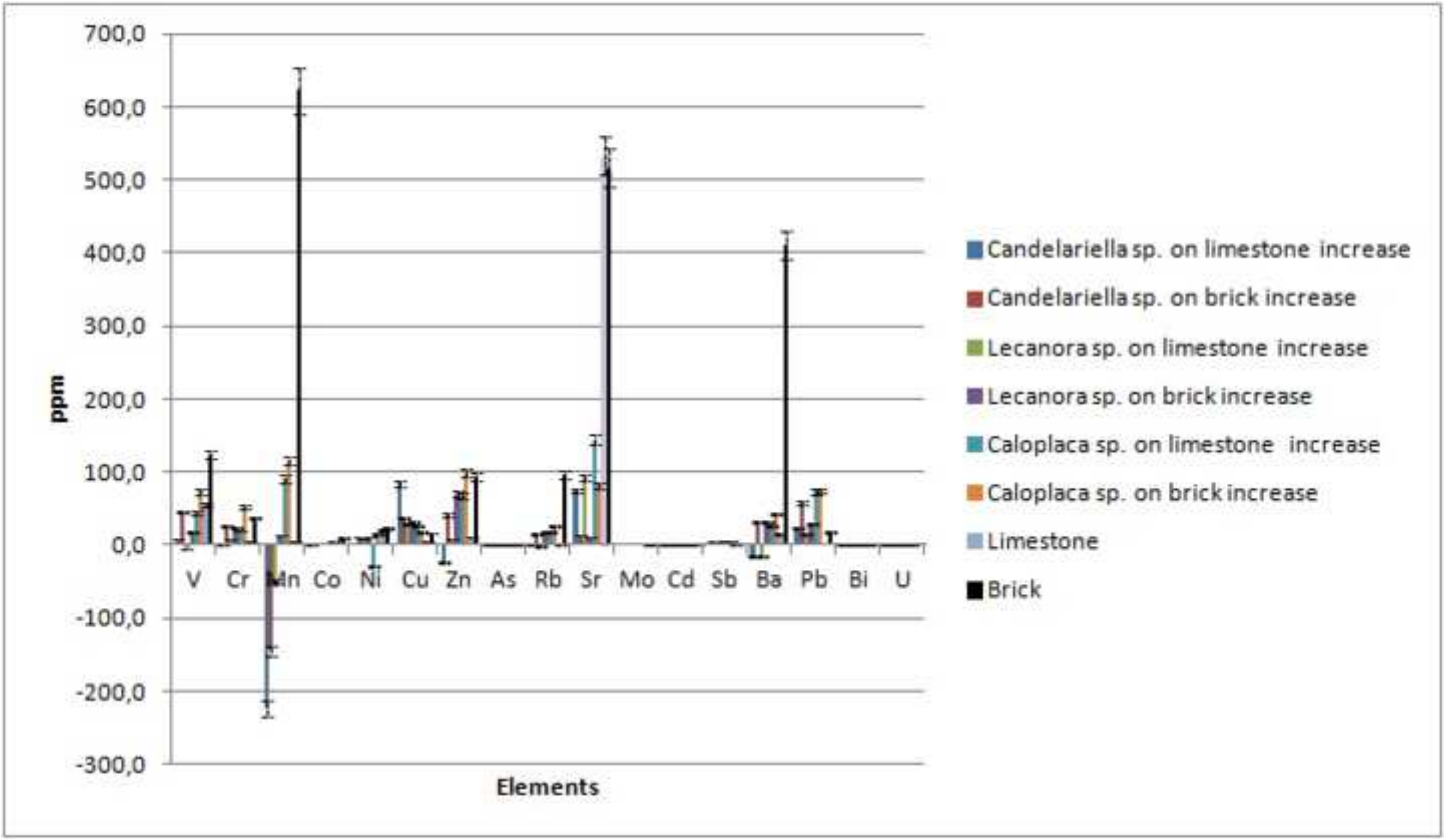
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