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Relatório de Projecto

Identificação da origem dos principais focos de poluição através do mapeamento espacial de isótopos de enxofre na área industrial de Sines

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Preâmbulo

O trabalho proposto em Julho para a bolsa de investigação Universidade de Lisboa / Fundação Amadeu Dias com o título *Identificação da origem dos principais focos de poluição através do mapeamento espacial de isótopos de enxofre na área industrial de Sines* decorreu de Setembro desse mesmo ano a Julho de 2011. Todo o processo decorreu dentro dos limites previstos e todos os objectivos propostos inicialmente foram cumpridos, até mesmo ultrapassados. Durante o trabalho a bolseira foi envolvida em todos os procedimentos de um projecto de investigação desde a elaboração de hipóteses ao tratamento e análise estatística e interpretação de resultados. Neste sentido, era também um dos objectivos que a bolseira encarasse com seriedade o projecto elaborado de tal forma a que o seu produto final fosse a elaboração de um relatório no formato de um artigo científico, de modo a que pudesse compreender o modo como são publicados os estudos em Ciência e qual o exercício de raciocínio necessário para tal. Assim, este relatório foi elaborado respeitando o formato de um artigo científico no que toca à estruturação do texto e à exposição e discussão dos resultados, mantendo-se, no entanto, os capítulos definidos pelo Regulamento das Bolsas Universidade de Lisboa / Fundação Amadeu Dias 2010/2011.

Agradecimentos

Gostaria de deixar um agradecimento especial à Sofia pela paciência que demonstrou ao longo deste ano e pelas gargalhadas durante o trabalho de campo; ao Pedro sempre pronto a ajudar e também aos seus conhecimentos de SIG, sem eles metade deste estudo teria sido praticamente impossível; à Cristina Antunes, à Silvana e ao Paulo Beliche pela ajuda prestada nas colheitas de campo; ao Rodrigo Maia do SIAAF pela sua enorme simpatia e disponibilidade; à Professora Cristina Máguas pela sua frontalidade e espírito crítico; a todos os restantes sempre presentes na salinha dos doutorandos, pela companhia e por tantos lanchinhos e celebrações; e por último, mas mais especial ainda, à Dra. Cristina Branquinho, por ter arranjado tempo no meio das suas milhares de tarefas, para mais um projecto e para guiar uma jovem e inexperiente recém-bióloga nos seus primeiros passos na investigação, por me ter sempre estimulado a fazer mais e melhor e pela sua incansável paciência para a minha falta de '*know-how*'.

Resumo

A monitorização do enxofre (S) é extremamente importante nos dias de hoje, mesmo tendo em conta a implementação e legislação de medidas de redução das emissões de compostos sulfurosos, já que é necessário averiguar a eficácia destas mesmas medidas e o modo como os valores de S nos ecossistemas têm vindo a evoluir. Esta monitorização pode ser feita com recurso a biomonitores, como é o caso dos líquenes. Dadas as características fisiológicas destes organismos, os líquenes retêm em si os elementos que absorvem da atmosfera e portanto a sua análise é representativa da mesma. No entanto, o S pode ser emitido a partir tanto de fontes naturais, como vulcânicas, marinhas, ou de processos de decomposição, como de fontes

antropogénicas, dificultando a identificação da sua origem. Para responder a este problema é feita a análise isotópica do S retido pelos líquenes, cuja assinatura é fiel àquela que interceptaram do ambiente e, por sua vez, nos dá a indicação da fonte emissora de onde o S proveio. Através da amostragem do líquen *Parmotrema hypoleucinum* na área industrial de Sines e envolventes, e sua análise em concentração de S e seus valores isotópicos, este estudo demonstrou que na área de estudo existem duas fontes principais de S: antropogénica e marinha. A influência marinha, traduzida por valores isotópicos superiores, estende-se até cerca de 4000m de distância ao mar, mas mesmo nesta faixa foi possível distinguir as fontes antropogénicas de S (ETAR e Central Termoeléctrica de Sines). Através da comparação dos resultados obtidos com resultados de estudos anteriores na mesma área de estudo, concluiu-se também que as emissões de S têm vindo a reduzir, o que significará que as medidas de mitigação implementadas pelas indústrias emissoras de S serão eficazes.

Framework

Lichens have been used as biomonitors for atmospheric pollution for more than 30 years, and several scientific articles and reviews have been published regarding this subject (Pirintsos, et al., 2003; Vingiani, et al., 2004). Lichens retain essential characteristics that make them excellent biomonitors: their morphology doesn't change with seasons, allowing accumulation to occur all year-round (Szczepaniak, et al., 2003); they have significant longevity (Sloof, 1993), therefore *in-situ* lichens can integrate the pollution for long-term or used to study the evolution of atmospheric conditions when collected over different periods of time (Garty, 2001; Wadleigh, 2003; Rosamilia, et al., 2004); because lichens lack a root apparatus, protective cuticle and stomata, they mostly depend on atmospheric inputs and accumulate mineral elements, such as heavy metals, far beyond their metabolic needs (Backor, et al., 2009), thus reflecting wet and dry atmospheric deposition (Vingiani, et al., 2004); lichens respond to changes in the environment within a few months (Cuny, et al., 2001; Wadleigh, 2003; Backor, et al., 2009) and they tend to reach concentration and isotopic equilibrium with the atmosphere within that time (Wiseman, et al., 2002; Wadleigh, 2003). Lichens use highly effective but unselective mechanisms to absorb and accumulate low concentration minerals present in air and moisture (Batts, et al., 2004), some species also being rather tolerant even to some high levels of trace elements (Garty, 2001; Häffner, et al., 2001; Van Dobben, et al., 2001), which allows their use to estimate trace element concentrations in the environment (Van Dobben, et al., 2001). These characteristics combined with their vast geographical dispersion and low costs of sample collection and treatment (Martin, et al., 1982) make them some of the best biological communities' biomonitors of air pollution (Garty, 2001).

From several air pollutants, sulphur dioxide (SO_2) seems to be the one which affects lichen survival and biodiversity the most (James, 1973), thus the mapping of lichen diversity has become a routine process in many countries (Sigal, 1988; Nimis, 1999) to easily assess the biological impact of air pollution due to SO_2. Hawksworth, et al. (1970) established and later revised (Hawksworth, et al.,

1976) a semi quantitative scale that correlates zones of mean SO_2 concentrations with the occurrence of certain lichen species.

However, despite that the decline in lichen biodiversity may indicate a prolonged exposure to air pollutants (James, 1973; Ferry, et al., 1973; Doll, et al.; Guderian, et al., 1985; Sigal, et al., 1983), it is crucial to correctly identify pollution sources, firstly because many of the anthropogenic pollutants such as SO_2 also have natural sources such as, sea spray, volcanoes emissions or decomposition processes (McArdle, et al., 1995; Chin, et al., 1996; Beričnik-Vrbovšek, et al., 2002; Hissler, et al., 2008), and secondly to apply adequate strategies to improve air quality. For that some researchers have been using the concentration of sulphur (S) measured in epiphytic lichens (Evans, 1996; Blake, 1998; Wadleigh, et al., 1999; Wiseman, et al., 2002; Wadleigh, 2003; Vingiani, et al., 2004) as an estimate of the amount of S that suffers deposition from the atmosphere. This is possible since changes in S concentration in transplant lichens have shown to be mainly attributable to changes in the inorganic S fraction and not in the organic S-fraction (Krouse, 1977; Takala, et al., 1985; Häffner, et al., 2001) and because epiphytic lichens depend mainly on atmospheric absorption. As already has been shown for several case studies, S concentrations seem to increase with decreasing distance from an anthropogenic source of S (Wadleigh, et al., 1999; Novák, et al., 2001; Vingiani, et al., 2004). Yet the S concentrations in lichens do not provide us the S source, because it is not possible to distinguish natural sources from anthropogenic sources exclusively through concentration measurements. However, it is possible to distinguish them when these are combined with their stable isotopic signatures (Wiseman, et al., 2002; Wadleigh, 2003; Hissler, et al., 2008).

Although much effort and great deals of money have been put into reducing the anthropogenic emissions of S in western countries, resulting in a decrease of its atmospheric concentration (Smith, et al., 2001; Aherne, et al., 2008; Hamed, et al., 2010), the monitoring of atmospheric S is still very important, not only because SO_2 induces great damage in the ecosystems, such as forest decline due to soil and water acidification (Ulrich, et al., 1980; Reuss, et al., 1987; Zhao, et al., 1998), but also because it is crucial to evaluate the effectiveness of the mitigation measures adopted. Sulphur isotopes ^{32}S and ^{34}S (the most abundant) are frequently used to study pathways through the ecosystems (Winner, et al., 1978). Their atmospheric values have been determined in many places given that they may hold source-specific information that can help identify S sources and assess their relative impact (McArdle, et al., 1995; Ohizumi, et al., 1997; Novák, et al., 2001; Xiao, et al., 2002; Pruett, et al., 2004). The natural abundances of the two isotopes are fairly constant (Beričnik-Vrbovšek, et al., 2002), but chemical, physical and biological processes lead to the enrichment, or depletion, of one isotope relatively to another (Lindberg, et al., 1986; Wadleigh, 2003; Batts, et al., 2004) leading to significant differences between different samples (Griffith, 2004). These variations, or fractionation, are expressed in terms of a 'delta' (δ) and, although small, they are characteristic of a certain emission (Lindberg, et al., 1986). Measuring absolute abundances of a certain isotope is

extremely unpractical, except for heavy metals, therefore it is easier to compare and analyse the isotopic ratios of samples, relatively to a known standard material (Beričnik-Vrbovšek, et al., 2002).

In respect to S, the $\delta^{34}S$ values of major contributors to the atmosphere can be divided into marine sources, usually with higher values, and continental sources, usually with lower values (McArdle, et al., 1995; Wadleigh, et al., 1999; Wiseman, et al., 2002). As for anthropogenic emissions, the $\delta^{34}S$ values can show a wide range, depending on the source (Wiseman, et al., 2002; Xiao, et al., 2010).

Lichens are extremely useful for identifying S pollution sources and Conti, et al. (2001) recognised that as biomonitors, lichens respond better to SO_2 than to other pollutants. Little or no fractionation occurs to $\delta^{34}S$ after it has been assimilated by this organism (Mektiyeva, et al., 1976; Trust, et al., 1992; Batts, et al., 2004; Backor, et al., 2009), except when the lichen is exposed to excessive atmospheric SO_2, where the selective emission of reduced S compounds causes an increase of $\delta^{34}S$ within the lichen (Batts, et al., 2004). Therefore, lichens retain the isotopic signature of the surrounding atmosphere (Krouse, 1977; Evans, 1996; Wadleigh, et al., 1999), integrating it for long term periods.

In contrary to the mapping of lichen diversity to infer pollution impacts, or its correlation to measured atmospheric pollution, the mapping of isotopic values in lichens, be it S or any other element, is not of common practise. Except for Jeran, et al. (1996), who mapped the concentration of trace elements measured in lichens and Wadleigh and Blake (1999), who mapped the $\delta^{34}S$ found in epiphytic lichens. Those were the only two studies that simultaneously mapped S isotopes and elemental concentrations. Moreover, Wadleigh and Blake (1999) could not relate the isotopic values with the S concentrations in lichens, and therefore they did not integrate the geographical analysis of S isotopes with the geographical analysis of its concentration, which greatly increases the chances of understanding S sources.

Nevertheless several authors have verified that coastal lichens may reveal two different marine-derived isotopic signatures: sea spray with +21‰, derived from the well-mixed reservoir of ocean sulphate (SO_4) (Rees, et al., 1978); and +15,6‰ ± 3,1‰ from dimethyl sulphide (DMS) (Calhoun, et al., 1991) a form of biogenic sulphur that is a major secondary metabolite in some marine algae. DMS can also be produced by bacterial transformation of dimethyl sulfoxide (DMSO), usually present in sewers where it can cause odour problems (Glindemann, et al., 2006). Although isotopic signatures from anthropogenic emissions vary greatly depending on the nature of the source (Wadleigh, 2003) values between ≈5‰ (pulp and paper mills) and ≈7‰ (thermal power plant) were obtained by Beričnik-Vrbovšek, et al. (2002) and Wadleigh, et al.(1999), respectively, from lichens near anthropogenic sources. Novák, et al. (2001) obtained a $\delta^{34}S$ value of 3,4‰ on forest floor samples (ecological sulphur pools) in Czech Republic, which only differed from the Czech Republican coal (main pollution source) by 1,4‰. Lichens, unlike forest floor, do not supply nutrients to other organisms; therefore they will probably be more faithful to the original atmospheric source of isotopic signature. The extent of the impact of the marine-derived isotopes was not previously

investigated and that is important to understand what the level of interference of those isotopes is when using S isotopes as environmental tracers.

Thus, when we have simultaneously natural and anthropogenic sources that change over space, as in the case of industrialized coastal areas, it is very important to distinguish between natural and anthropogenic S sources and evaluate the amount of atmospheric deposition due to S emissions. Since the 80's that European legislation has been imposing strong emission reductions to the industry and it is important to evaluate whether those reductions have been reflected at the ecosystem level.

Objectives

This study aims to apply the great utility of lichens as biomonitors to spatially trace S pollution sources, integrating S concentration and isotopic analysis in order to distinguish between natural and anthropogenic sources of S in a Portuguese coastal and industrial area. We also aim to compare the level of S that was intercepted by lichens in this study with the one obtained in previous studies in the region in order to evaluate the impact of the reduction in atmospheric emissions.

Applied Methodology

Study area

The study area, with approximately 1500km^2 (50x30km), is located in the SW the coast of Portugal, having the Atlantic ocean on its western side. It comprises two small mountain ranges (Serra de Grândola, 383m; and Serra do Cercal, 378m) with N-S orientation, parallel to the coast (Fig. 1a).

The annual average temperature is 16 to 17,5°C, the average annual precipitation is 600 to 1000mm and the average annual insolation is 3000h (averages from 1931 to 1960; source APA,2004). The dominant winds come from the N-NW direction.

Figure 1b shows the land use cover in the study area. Four municipalities are included in the area of study: Grândola (population 14.901); Santiago do Cacém (population 31.105), Odemira (population 26.106) and Sines (population 13.577) (INE, 2001). The area comprises a number of important industries established since the late 1970's: a coal-fired power station, an oil refinery, a petro-chemical plant, an industrial sewage treatment plant and, more recently, an industrial landfill, and many other smaller industries. There is also the sea harbour of Sines, which is the main door for energetic supplies (coal, oil and derivatives, gas, and other chemicals) of the country, and an important harbour for general and container cargoes, with potential international importance. There are also two main motorways, which respond to the high vehicle traffic which is growing in importance over time, and a railway. Urban development of littoral towns has recently (last 30 years) expanded, but agro-forest activities (mainly cork-oak woodlands – *Quercus suber* L.) rule the inland landscapes. The natural reserve area Parque Natural do Sudoeste Alentejano e Costa Vicentina (PNSACV) is located in the SW.

Sampling

Lichen thalli of the species *Parmotrema hypoleucinum* (Steiner) Hale were selected for this study due to its ubiquitous distribution and tolerance to a variety of land uses, and because it has been used during the last years to monitor a set of pollutants in the same study region (Augusto, et al., 2010). Samples were collected on the 7th of February in 2011 in 43 points (point no. 37 was doubled), distributed in order to cover as many S sources as possible: sea influence (NE and SE points), urban influence (points close to the three urban centres indicated), industrial influence (points close to the industries indicated) and agro-forestry management influence (NW and W points) (Fig.1b).

Sulphur concentration and isotopic analysis

All samples were collected from either pine trees (*Pinus* spp.) or cork oaks (*Quercus su*ber L.), except for samples no.10 (olive tree, *Olea europaea* L.), 22 (*Viburnum tinus* L.), 39 (garden tree) and 41 (dune vegetation, *Corema album*), and sample coordinates were later registered using *Google Earth* (*Google*), which was also used to estimate the elevation of each sampling point and its linear distance to the sea. An effort was made to collect around 10g for each sampling point. Each sample came preferentially from at least 5 close trees, however when not possible only one tree was sampled. Also, lichens from dead trees and lichens positioned less than one meter from the ground were avoided.
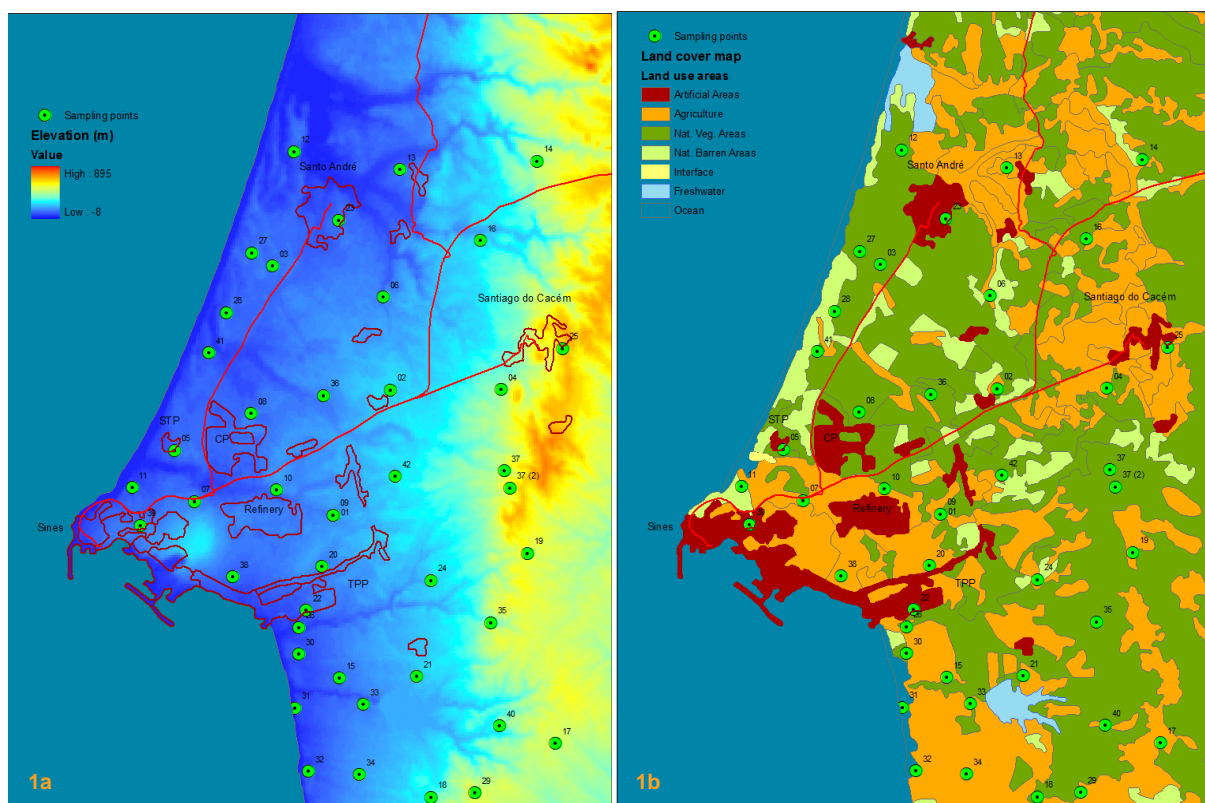

Figure 1a – Elevation map of the area of study.
Figure 1b – Land cover map of the area of study.
Red lines represent the main motorways and *Artificial Areas,* and the sewage treatment plant (STP), the chemical plant (CP), refinery and the Sines Thermal Power Plant (TPP) are indicated.

Samples were cleaned from tree bark fragments and dried at room temperature, to be later powdered and homogenized in a Reitsch MM2000 ball-mill (in FCUL, laboratory 2,5.39). From the total 10g collected, the samples were homogenised and 3mg were weighted for S concentration and isotopic analysis. These were analysed for all samples (n=43) using continuous flow isotope mass spectrometry (CF-IRMS) (Preston, et al., 1983) on a Isoprime (GV, UK) stable isotope ratio mass spectrometer, coupled to an EuroEA (EuroVector Italy) elemental analyser for online sample preparation by Dumas-combustion which is located in FCUL in the SIAF Lab (2.1.16). Regarding $\delta^{34}S$, the standards used were IAEA-S1 and IAEA-NBS127 and results were referred to Viena Canyon Diablo Triolite.

Not all samples were replicated due to analytical costs. However at every 9 samples, one sample was replicated three times in order to guarantee measurement accuracy, and the maximum percentage of variance between each of those four repeated samples was 1,9% for %S and 4,4‰ for $\delta^{34}S$, the minimum was 0,9% for %S and 1,4‰ for $\delta^{34}S$, and the mean percentage of variance was 1,6% for %S, and 2,6‰ for $\delta^{34}S$ .Values from 9 replicates of laboratory standard material interspersed among samples in every batch analysis were used to calculate the precision of the isotope ratio analysis, which resulted in 0.35‰.

Statistical analysis and sulphur mapping

All data from the elemental and isotopical analysis was organized using *Microsoft Excel* (*Microsoft*), combined with the sample coordinates and integrated with land use data with *ArcMap v9.3* (*ESRI Inc.*). To produce S concentration and isotopic signature maps both %S and $\delta^{34}S$ values obtained were mapped and interpolated with Inverse Distance Weighted (IDW) interpolation, using *ArcMap v9.3*.

However, given that different S sources lead to differences in its dispersion length, buffers of 100, 200, 300, 500, 1000, 2000, 3000 and 5000m were drawn around each sample point and intersected with the CORINE Land Cover 2006 map of Continental Portugal. The different land use areas inside each buffer were calculated using *ArcMap v.9.3* and exported to *Excel* for data organization: land use areas that referred to artificial areas, such as urban and industrial areas and roads, were aggregated as *Artificial Areas*; any agricultural, silvicultural and pasture systems were aggregated as *Agriculture*; natural forests and any other naturally vegetated areas were aggregated as *Natural Vegetation Areas* (*Nat. Veg. Areas*); burnt forest areas, rocky areas, degraded or recently cut/planted vegetation and areas with sparse vegetation were aggregated as *Natural Barren Areas* (*Nat. Barren Areas*); wetlands and intertidal zones were aggregated as *Interface*; freshwater bodies were aggregated as *Freshwater*; the ocean and beaches were aggregated as *Ocean*.

Normality of all data was analysed using the Shapiro-Wilk (W) normality test available in *Statistica*. Correlations and linear and multiple regressions between %S, $\delta^{34}S$, and land use were also calculated for each buffer using *Statistica*. Since most variables didn't show a normal distribution, Spearman correlations (ρ) were used (Table 1a and 1b in annexes). The best correlation values

between the different land use types and buffers, and both %S and δ^{34}S defined which variables would be later included in a Principal Components Analysis (PCA). A logarithmic regression between δ^{34}S and distance to sea was also calculated to establish the maximum distance of influence (MDI) of the sea on δ^{34}S values, which was observed graphically as the point from which δ^{34}S values remained constant.

A Principal Components Analysis (PCA) was used to observe the relationship between the different types of land use and both %S and δ^{34}S. As already mentioned, the land use variables were correlated to %S and δ^{34}S, considering each separate buffer. Those included firstly in the PCA were those which presented the best Spearman correlation values with either %S or δ^{34}S (see Table 1a and 1b in annexes). These were: *Artificial Areas* with the 300m buffer, *Nat. Veg. Areas* with the 100m buffer, *Nat. Barren Areas* with the 100m buffer and *Ocean* with the 2500m buffer. *Agriculture* was also included but the buffer selected did not correspond to the one with higher correlations (see Table 1a and 1b in annexes), because it was considered that this high correlation did not reflect the true effect of agricultural areas of %S, or δ^{34}S, which is usually felt in a smaller radius. Elevation was also included as a variable since it showed significant correlations between both %S and δ^{34}S. However, in a second instance redundant variables were removed from the final PCA. The removed variables were *Artificial Areas*, *Agriculture*, *Nat. Veg. Areas* and *Nat. Barren Areas*, leaving *Ocean* and *Elevation*, together with %S and δ^{34}S. The factor scores of the component which better explained the variance of the variables were exported to *Excel* where the highest absolute value was summed to the rest in order to transform values into positive. These transformed factor scores were then exported to *ArcMap v9.3* and interpolated with an IDW interpolation, in order to obtain a land use influence map.

Given that the effect of *Artificial Areas* did not appear as a relevant component due to the strong influence of the sea on these parameters another strategy was implemented. An ocean influence model was estimated through a linear regression between the δ^{34}S values and their distance to the sea up to 4000m from the coast (MDI) since this was shown to be under maritime influence. Once the most important trend was obtained we registered the residuals from this model as were reflecting the deviations from the general model trend, being that negative residual values were probably resultant from anthropogenic influences, and positive residual values were probably resultant from a higher ocean influence than the predicted by the model. These residuals were then interpolated in *ArcMap v9.3* with an IDW interpolation, so that the deviation from the ocean influence model could be obtained.

Results

Sulphur concentrations in lichens over time

The mean, median, minimum, maximum and standard deviation values for S concentrations (expressed in %) and isotopic S values (expressed in ‰) measured from 43 lichen samples are

presented in Table 2 (in annexes). Sulphur concentrations varied from 0,06% to 0,19%, with a mean value of 0,11.

Lichen transplant studies to the polluted city of Naples showed an increase of S concentration values to 0,261% for the species *Phagnum capillifolium* (Ehrh.) and 0,225% for the species *Pseudevernia furfuracea* (L.) Zopf (Vingiani, et al., 2004). Much lower S concentration values were obtained in the island of Newfoundland, Canada, for the lichen *Alectoria sarmentosa* (Ach.) Ach. (0,026% in interior locations away from industrial S sources, to 0,115% in S point sources). However, the lower S concentration values obtained by Wadleigh, et al. (1999) may be due to the fact the lichen *A. sarmentosa* tends to have lower S concentrations relatively to other lichens (Evans, 1996; Blake, 1998; Wadleigh, et al., 1999), or that it occurs in very pristine areas.

Only few studies published in international journals used *P. hypoleucinum* to measure pollutants concentrations (Pinho, et al., 2008; Augusto, et al., 2010) and none of these showed to have measured specifically to measure S concentrations and, or, isotopic values. However, some
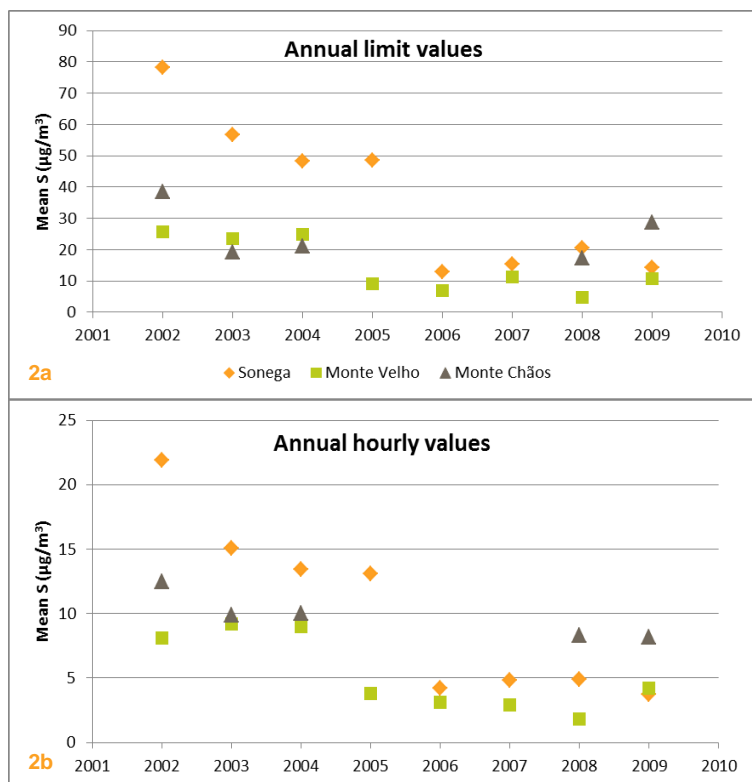
Figure 2a – Annual mean limit values of S measured from 2002 to 2009 in three air quality stations in the area of study (data source APA, 2010).
Figure 2b – Annual mean hourly values of S measured **from 2002 to 2009 in three air quality stations in the area of study (data source APA, 2010). The same legend of Figure 2a applies to Figure 2b.**

reports from studies developed in the same region during the SINESBIOAR project, led by the Commission for Regional Coordination and Development of the Alentejo (CCDR- Alentejo) and financed by the European Union through the LIFE Environment programme, have shown to measure %S in the same lichen species. In fact, when we compared the values obtained for S in the present study with the ones reported in 2002 we found that for the same area of study the values decrease approximately by 50% in this study (see Table 2 in annexes). This is very interesting since that new legislation implemented between 2002 and 2011 lead the local industries to reduce considerably the SO_2 emissions as a result of large investments in technological solutions to reduce this pollutant. To confirm that the concentration of S in lichens reduced due to the reductions in air quality in the region we plotted the SO_2 concentrations measured in the 3 air quality monitoring stations from 2002 to 2009, which that are located in the studied region (source APA, 2010). In fact we observe a considerable decrease in the levels of SO_2 for all the air quality monitoring stations (Fig. 2a and b) especially in the one located in the direction of the prevailing winds south from the industrial region (Sonega). The decrease in SO_2 in air was more than 50%, and was reflected in the

decrease observed in lichens. This could be interesting to explore since the effects in ecosystems do not take the same time to respond as the atmospheric levels of SO_2, showing that lichens might reflect some kind of memory of the highest emissions that occurred in the region. Revisiting some of these sampling sites in the future, would be interesting to track the pattern of decrease of S
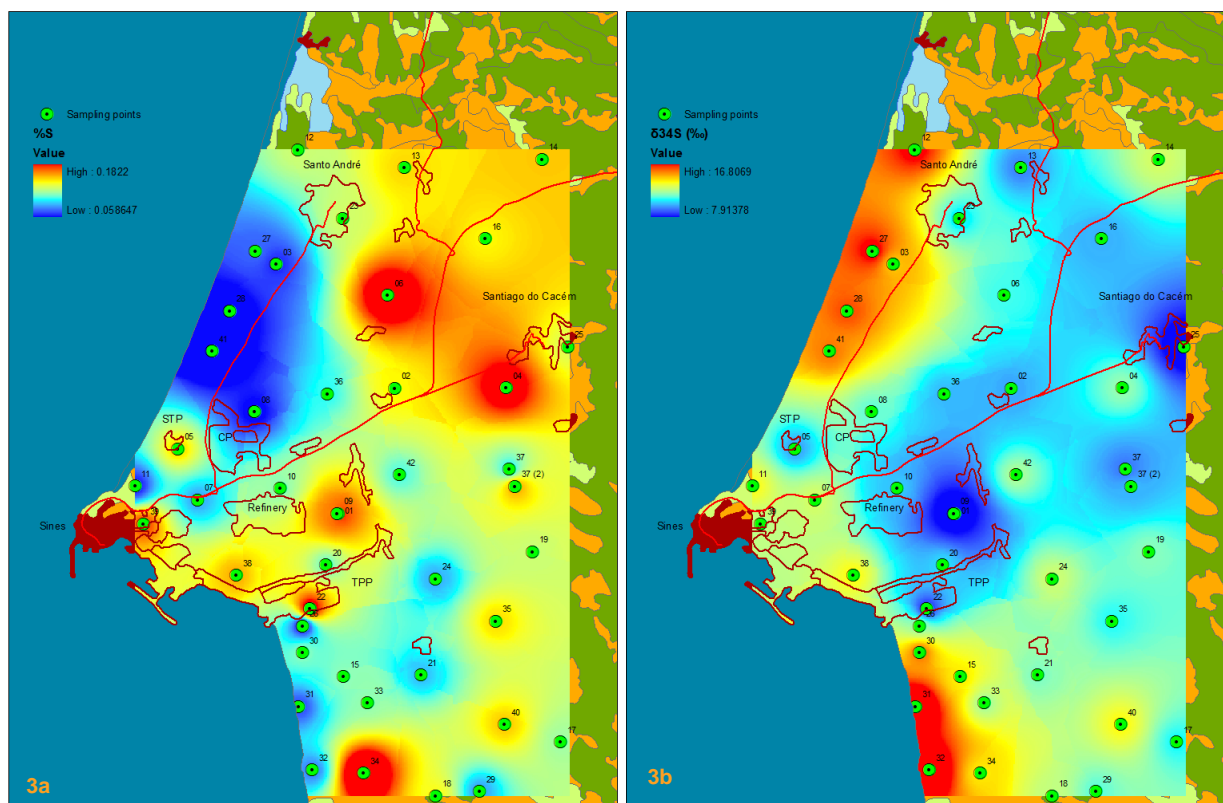

Figure 3a – Inverse Distance Weighted interpolation of the %S concentration values measured in lichens (n=43).
Figure 3b – Inverse Distance Weighted interpolation of the δ^{34}S values measured in lichens (n=43).
Red lines represent the main motorways and *Artificial Areas*, and the sewage treatment plant (STP), the chemical plant (CP), refinery and the Sines Thermal Power Plant (TPP) are indicated.

concentration in lichens over time.

The range of δ^{34}S in the studied area

As for the δ^{34}S values a wide range according to S source type was found in this study: δ^{34}S varied from 7,91‰ to 16,81‰, with a mean value of 11,46‰. These values are in accord with those from Wadleigh, et al. (1999) and by Novák, et al. (2001), who obtained a maximum of ≈ 16‰ for δ^{34}S values obtained in coastal areas and concluded that these were related to the ocean influence. In the same study, Wadleigh, et al. (1999) obtained minimums of ≈ 5‰ from sampling points near pulp and paper mills, values which they related to S industrial emissions.

Beričnik-Vrbovšek, et al. (2002) also studied the S isotopic signatures near SO_2 anthropogenic sources and obtained δ^{34}S values around 7‰ in samples of *Hypogymnia physodes* (L.) Nyl. exposed to SO_2 originating from a nearby power plant.

Sulphur sources

Figures 3a and 3b show the IDW interpolations of the S concentrations and isotopic values in lichens. When observing Figure 3a we can see that lower values of %S are dominant in the NW, an

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area more exposed to the sea, and increase towards the SE direction. Values in SW are not as low and this area is not as exposed either, receiving the NW winds from the Artificial Areas in red, which present higher values of %S. The higher %S values shown in red in points 04, 06 and 34 maybe due to several causes: 04 is very near to the main motorway that connects Sines, first to Santiago do Cacém, and then to the freeway to Lisbon; 06 is between the motorway just mentioned and the one that connects Sines to Santo André; 34 is probably receiving the S emitted by the Sines Thermal Power Plant, which has one of the tallest chimneys in Europe (234m) and therefore, the S emitted may not be recorded between point 34 and the points which are closest to it.

As for Figure 3b, δ^{34}S shows a clearer pattern than %S. The highest values (near 16‰) were measured in the coast line (NW and SW) and are a result of strong ocean influence (discussed further). Lowest values fall near the *Artificial Areas*: Santiago do Cacém urban area and the industrial area of Sines, the last with three low peaks one above the Sines Thermal Power Plant (09, 01), another to south (22 and 26), and the third near the sewage treatment plant (05) that deals with sewage from the petrol industry. The urban areas of Sines and Santo André show neither characteristically sea isotopic signatures, nor characteristically anthropogenic ones, probably resulting in the mixture of these two.

In Figure 4 we can see that the effect of the sea on δ^{34}S is observed up to 4.000m from the sea. After this distance the values decrease more slowly with increasing distance from the sea. Thus, it appears that the maximum distance of influence (MDI) of the sea on δ^{34}S values is around 4.000m (Fig. 4). This an important result, since if we want to use δ^{34}S as a tracer for environment studies up to 4km from the coast we need to be careful when interpreting data due to the interference of the ocean signature.

As already mentioned, %S and δ^{34}S and selected variables of land use were confronted using a PCA (Fig. 5). The land use variable which has shown better correlation with either %S or δ^{34}S and the less redundancy in the PCA was *Ocean* considering the 2.500m buffer, which means that the maximum ocean influence is at a distance of 2.500, although its influence reaches the 4.000m (MDI). *Elevation* was also included not only because it showed a significant correlation with δ^{34}S (ρ=-0,431; p<0,05), but because it may explain the δ^{34}S gradient in the study area (see Fig. 6), which is traversed N-S by the Serra de Grândola and Serra do Cercal (see Fig. 1), two mountain ranges parallel to the coast.
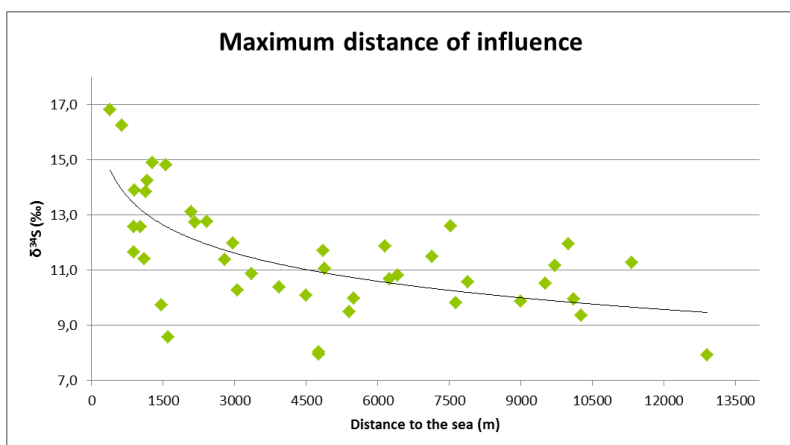

Figure 4 - Scatterplot showing the δ^{34}S values and their correspondent distance to the sea (n=43). The distance from which δ^{34}S stabilizes (4000m) was considered to be the maximum distance of influence (MDI) of the sea on δ^{34}S values in lichens.

In Figure 5 %S appears in opposite of both δ^{34}S and *Ocean* regarding the first factor (58,20% total variance), whose factor scores were used to interpolate

the ocean influence in the area of study (Fig. 6). The eigenvectors for %S and for δ^{34}S are 0,387 and -0,563, respectively. The *Ocean* land use variable for the 2.500m buffer appears highly related to δ^{34}S, as expected from the high correlation between them (see Table 1a and 1b in annexes) and its eigenvector for the first factor is -0,585. Therefore in Figure 6 areas shown in red stand for higher %S, lower δ^{34}S and less ocean influence, while areas shown in blue stand for lower %S, higher δ^{34}S and higher ocean influence. According to Rees, et al. (1978) sea spray usually presents an S isotopic composition of +21‰ and can raise to +30‰ during the spring and summer plankton productivity peaks (McArdle, et al., 1995), but Wadleigh and Blake (1999) obtained a maximum of +16,‰ from lichens in the coastal areas of the island of Newfoundland, Canada, which is in accord to the DMS isotopic signature which is +15,6‰ ± 3,1‰ (Calhoun, et al., 1991).
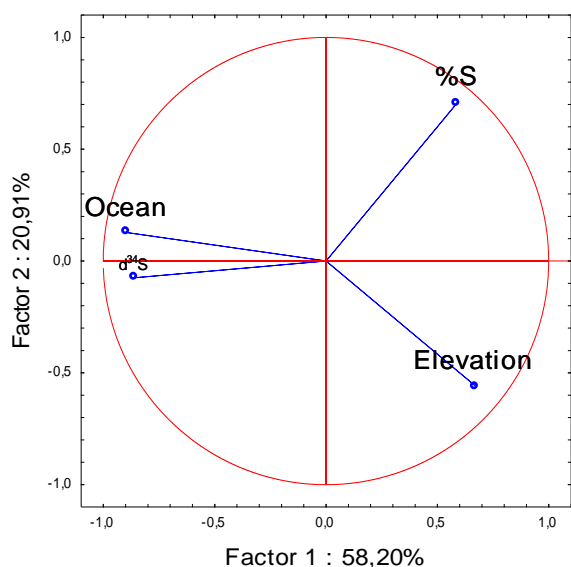

Figure 5 - PCA ocean Principle Components Analysis (PCA) showing the ocean influence and its close relation to δ^{34}S (n=43 in all variables). The *Ocean* land use variable corresponds to the 2500m buffer.

Figure 6 clearly shows the predominant effect of the sea in the NW and SW, characterized by lower values of %S and higher values of δ^{34}S (points 03, 27, 28, 41, 42 and 31, 32). This effect seems to be reduced in the SE direction, possibly due to the direction of predominant winds (NE).

Areas with higher %S values and lower δ^{34}S values (in red and orange) appear to be related to *Artificial Areas* (drawn in red line), such as Santiago do Cacém and the industrial area of Sines.

Intermediate values (in yellow and light blue) possibly represent the mixing of S from the marine environment with that from anthropogenic sources, similarly to the results obtained by Wadleigh, et al. (1999). These are unlikely to represent background levels from biogenic emissions, since these are thought to average less than 2‰ of total annual emissions (Nriagu, et al., 1987).
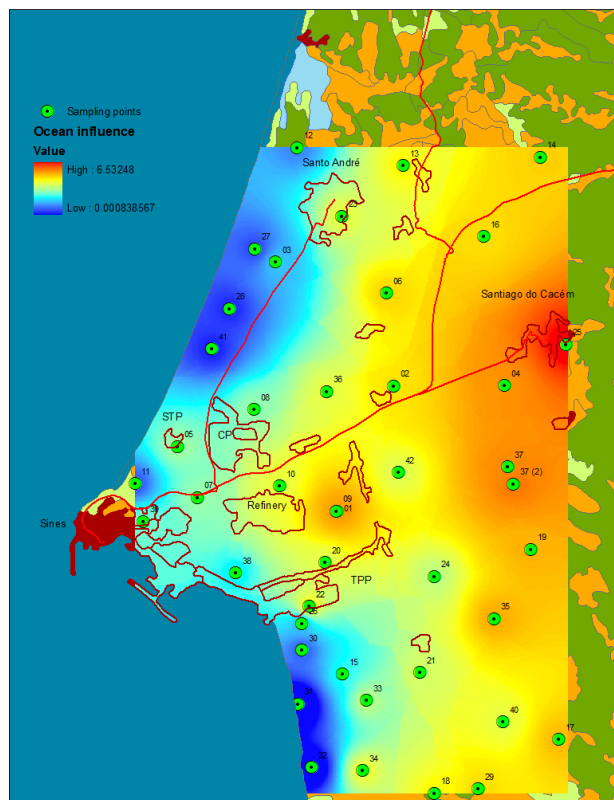

Figure 6 - Inverse Distance Weighted (IDW) interpolation of the PCA factor scores of the first component (Fig. 5), showing the ocean influence in the area of study.
Red lines represent the main motorways and Artificial Areas, and the sewage treatment plant (STP)

As for the urban areas of Sines and Santo André, due to the fact that the first is surrounded by the sea and the second is very near to it, the strong influence of the sea possibly dilutes the characteristically lower urban S isotopic signature (Santo André) and may even

predominate over the last (Sines).

Our results point to the same conclusions as those from Wadleigh, et al. (1999) and Beričnik-Vrbovšek, et al. (2002): higher $\delta^{34}S$ values (and lower values of %S) are a result of the ocean influence which is reduced in direction to the interior and seems to be not so preponderant when other possible sources are present. The same $\delta^{34}S$ pattern has also been verified by air samples by McArdle, et al. (1995) who obtained $\delta^{34}S$ values of +5‰ and +6‰ for anthropogenic S sources and +22‰ values for marine S sources.
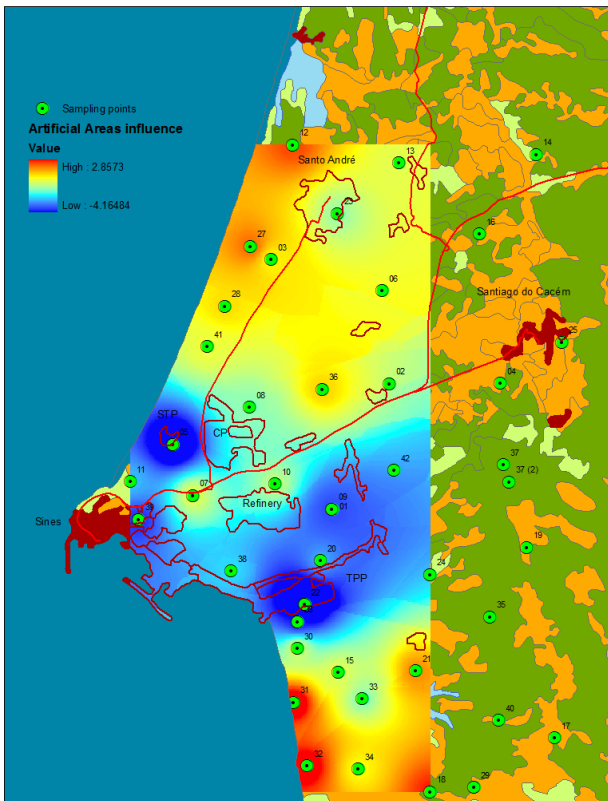

Figure 5 – Inverse Distance Weighted (IDW) interpolation of the residuals of an ocean influence model, showing the Artificial Areas influence in the area of study.
Red lines represent the main motorways and Artificial Areas, and the sewage treatment plant (STP)

The *Artificial Areas* land use influence is better expressed in Figure 7. As explained before, this map is the result of the interpolation of the residuals of an ocean influence model. Thus, in this map the ocean influence was removed and this allowed the revealing of *Artificial Areas* land use influence on $\delta^{34}S$. As we can see in Figure 7 the lower residuals fall mainly into two S point sources: the sewage treatment plant (point 05) and the Sines Thermal Power Plant (points 22 and 26). Interestingly, the S concentration values near the sewage treatment plant are not especially high, but nevertheless its isotopic signature reveals it as an anthropogenic source, which happens to be responsible for a strong odour that leads to some discomfort of the surrounding populations. On the other hand, Sines Thermal Power Plant shows a strong anthropogenic influence which is in accord with both the S concentrations found in points 22 and 26, and their isotopic signatures.
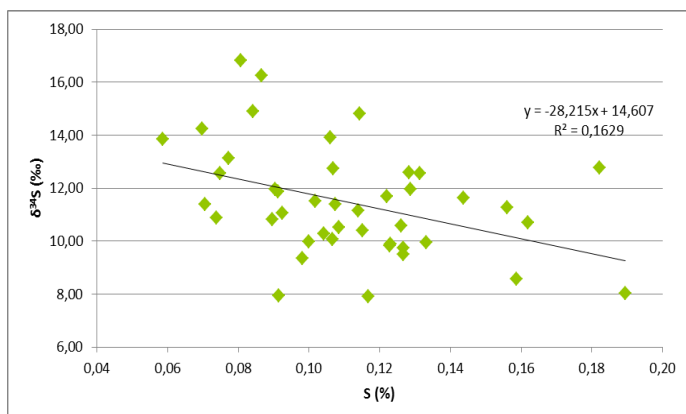
Lower but still anthropogenic influences are also found around the urban area of Sines and across the industrial area.

Sulphur concentration and isotopic signature

Figure 8 shows that the concentration of S in lichens is significantly related to the measured $\delta^{34}S$ values (r^2=0,163; p=0,007) and for higher values of S concentration in lichens, lower values of $\delta^{34}S$ were measured. Only a few studies have shown the same correlation both in lichens (Case, et al., 1980; Takala,

Figure 6 - Linear regression between %S and $\delta^{34}S$ measured in lichens (n=43). The linear regression equation and r^2 shown in the figure are significant for p<0,05.

et al., 1991) and leaves of superior plants (Case, et al., 1980; Krouse, et al., 1981) and the majority

of the published studies found does not relate S concentrations with their isotopic values. In this study S concentration and isotopic values were measured in a well distributed sampling area and different isotopic signatures were obtained (see Table 2 in annexes for maximum and minimum values), their linear relation with S concentration in lichens is extremely important for biomonitoring purposes as it greatly increases the accuracy of S source identification.

Budget Accomplishment

The budget plan proposed at the beginning of this project was entirely fulfilled.

The sampling of lichens was completed in a shorter time then the expected four days (only one day), although two more people were recruited for this task, in a total of six people (including the scholarship holder).

The preparation of lichen samples for elemental and isotopic analysis was carried by the scholarship holder, who also analysed the results with the aid of her tutor.

The concentration and isotopic analysis of S, more than 30 samples were analysed, but even so the analysis of the 43 samples didn't exceed the predicted 1.000€ budget for this task.

As for the congress participation proposed, a poster will be presented at the 9ISEG - International Symposium on Environmental Geochemistry in July 2012.

Conclusions

The results demonstrated a strong relation between %S and δ^{34}S, which allowed to draw further conclusions on the different pollution sources of the area of study. The estimated the MDI of the sea (Fig. 4) and the interpolation of the Ocean land use influence (Fig. 6) permitted a better understanding of the characteristics of the ocean influence on δ^{34}S measured in lichens and its range and pattern in the area of study. This range prolonged itself until 4000m to the coast, which means that bellow this distance from the sea the interpretation of δ^{34}S values must be conducted with care, and ocean influence must first be distinguished in order to identify other S sources, such as anthropogenic S point sources.

Furthermore, through isotopic analysis we were capable of 1) distinguishing anthropogenic effects in an environment where these to influences co-exist, but which is dominated by a marine isotopical influence (Fig. 7) and 2) trace different S point sources like the sewage treatment plant and the Sines Thermal Power Station. Surveys of lichen S content can serve as a warning system to locate pollution point sources when a correct integrated analysis of S concentration and δ^{34}S is carried so natural sources of S can be distinguished from anthropogenic ones.

This study also aided in the evaluation of the effectiveness of mitigation strategies that have been imposed on industries by European legislation on atmospheric emissions. As seen in Figures 3a and 3b, the S atmospheric emissions measured in three air quality stations located in the studied area have decreased from 2002 to 2009, and the same was observed when comparing lichens

collect in this area in 2002 with our results, which indicates that lichens also verified the success of the mitigation measures. Thus air quality monitoring can be achieved with biomonitors such as lichens as long as we bear in mind that these retain some memory of the past S emissions, since the decrease was not as strong as the one verified in air quality stations.

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Abbreviations

CP: (petro)Chemical Plant

IDW: Inverse Distance Weighted (interpolation)

MDI: Maximum Distance of Influence

STP: Sewage Treatment Plant

TPP: (Sines) Thermal Power Plant

29 de Julho, de 2011,
      A Bolseira,   A Tutora,

Annexes

	%S	δ^{34}S	Elevation
%S	1,000	-0,385	0,365
δ^{34}S	-0,385	1,000	-0,431
Elevation	0,365	-0,431	1,000

		Artificial Areas	Agriculture	Nat.Veg. Areas	Nat. Barren Areas	Interface	Freshwater	Ocean
Buffer 100m	%S	0,319	0,276	-0,170	-0,331	----	----	-0,250
	δ^{34}S	-0,323	-0,140	-0,041	0,308	----	----	0,365
	Elevation	-0,056	0,280	-0,046	-0,091	----	----	-0,318
Buffer 200m	%S	0,282	0,233	-0,165	-0,243	----	----	-0,250
	δ^{34}S	-0,348	-0,078	-0,063	0,276	----	----	0,365
	Elevation	-0,048	0,199	0,050	-0,088	----	----	-0,318
Buffer 300m	%S	0,281	0,185	-0,159	----	----	----	-0,232
	δ^{34}S	-0,422	-0,137	-0,029	----	----	----	0,421
	Elevation	-0,031	0,176	0,069	----	----	----	-0,370
Buffer 500m	%S	0,212	0,156	-0,065	-0,275	0,137	-0,099	-0,286
	δ^{34}S	-0,420	-0,081	-0,075	0,297	-0,187	-0,025	0,330
	Elevation	-0,206	0,103	0,265	-0,126	-0,137	0,081	-0,383
Buffer 1000m	%S	0,134	0,217	-0,028	-0,197	0,137	-0,041	-0,433
	δ^{34}S	-0,343	0,032	-0,179	0,096	-0,187	0,118	0,610
	Elevation	-0,235	-0,016	0,444	-0,197	-0,137	-0,124	-0,586
Buffer 2000m	%S	0,004	0,304	0,004	-0,195	-0,122	0,024	-0,319
	δ^{34}S	-0,238	-0,101	-0,239	-0,006	0,011	0,223	0,643
	Elevation	-0,437	0,077	0,550	-0,117	-0,254	-0,092	-0,739
Buffer 2500 m	%S	0,015	0,322	-0,003	-0,194	-0,014	0,088	-0,338
	δ^{34}S	-0,206	-0,111	-0,324	-0,060	0,034	0,259	0,670
	Elevation	-0,417	0,143	0,580	-0,062	-0,222	0,064	-0,764
Buffer 3000m	%S	0,016	0,314	0,016	-0,217	-0,006	-0,035	-0,372
	δ^{34}S	-0,179	-0,123	-0,359	-0,082	0,039	0,260	0,657
	Elevation	-0,472	0,181	0,569	0,011	-0,213	0,033	-0,798
Buffer 5000m	%S	-0,090	0,292	0,210	-0,155	-0,153	-0,031	-0,382
	δ^{34}S	-0,059	-0,163	-0,456	-0,211	0,088	0,341	0,573
	Elevation	-0,635	0,261	0,716	0,141	-0,393	-0,111	-0,839

		Mean	Median	Min.	Max.	Std.Dev.
	%S	0,11	0,11	0,06	0,19	0,03
	δ^{34}S (‰)	11,46	11,28	7,91	16,81	2,06
	Distance to sea (m)	4727	4500	390	12900	3484
	Elevation (m)	11	11	8	17	2
	%S SINESBIOAR	0,20	0,20	0,11	0,34	0,04
Buffer 100m	*Artificial Areas*	2143	0	0	31375	7571
	Agriculture	15022	12621	0	31375	14647
	Nat. Veg. Areas	11526	949	0	31375	14004
	Nat. Barren Areas	1673	0	0	31375	6124
	Interface	0	0	0	0	0
	Freshwater	0	0	0	0	0
	Ocean	1010	0	0	31375	5085
Bu	*Artificial Areas*	8424	0	0	125581	27390

Buffer	Class					
	Agriculture	58421	68130	0	125581	51350
	Nat. Veg. Areas	47775	28650	0	125581	49022
	Nat. Barren Areas	7037	0	0	103523	20107
	Interface	0	0	0	0	0
	Freshwater	0	0	0	0	0
	Ocean	3925	0	0	117851	19407
Buffer 300m	Artificial Areas	19580	0	0	282473	59503
	Agriculture	124848	153126	0	282618	103462
	Nat. Veg. Areas	112368	84217	0	282618	102124
	Nat. Barren Areas	0	0	0	0	0
	Interface	0	0	0	0	0
	Freshwater	0	0	0	0	0
	Ocean	8478	0	0	250120	41565
Buffer 500m	Artificial Areas	59862	0	0	691282	150411
	Agriculture	314932	294785	0	785191	242817
	Nat. Veg. Areas	325901	280610	0	785191	255953
	Nat. Barren Areas	59635	0	0	466300	124310
	Interface	79	0	0	3399	518
	Freshwater	282	0	0	12135	1851
	Ocean	24500	0	0	625192	104663
Buffer 1000m	Artificial Areas	246579	0	0	1798283	477296
	Agriculture	1122623	1155013	0	3141177	809608
	Nat. Veg. Areas	1380144	1348148	0	4834779	1045274
	Nat. Barren Areas	258997	10090	0	1299632	409488
	Interface	3754	0	0	161401	24613
	Freshwater	25603	0	0	693349	115522
	Ocean	143846	0	0	1943034	391559
Buffer 2000m	Artificial Areas	980144	281967	0,0	4745497	1299281
	Agriculture	4383655	4698124	224634,2	11469454	2555905
	Nat. Veg. Areas	5054897	4724882	0,0	10230278	3035054
	Nat. Barren Areas	902596	543767	0,0	3702782	960390
	Interface	13231	0	0,0	252929	54423
	Freshwater	128990	0	0,0	2306455	455466
	Ocean	1087356	0	0,0	7074522	1803191
Buffer 2500m	Artificial Areas	1515372	556332	0,0	6122079	1821533
	Agriculture	6766093	7165662	305303,1	16703459	3664552
	Nat. Veg. Areas	7767372	7523008	204649,4	14489149	4288052
	Nat. Barren Areas	1383413	991102	0,0	4795484	1320130
	Interface	20963	0	0,0	252929	67300
	Freshwater	177691	0	0,0	2362180	545801
	Ocean	2003008	0	0,0	10602902	2996130
Buffer 3000m	Artificial Areas	2140943	1331263	0,0	8666614	2423539
	Agriculture	9481947	10343858	354360,6	20971776	4794971
	Nat. Veg. Areas	11151192	10796275	482840,0	20373986	5905144
	Nat. Barren Areas	1935407	1546843	43115,6	6124098	1680594
	Interface	23528	0	0,0	252929	74337
	Freshwater	254892	0	0,0	2497195	656321
	Ocean	3283880	0	0,0	14706302	4567424
Buffer 5000m	Artificial Areas	5008658	3614970	0	15645219	4588746
	Agriculture	24486565	25285472	2454661	49504200	9957878
	Nat. Veg. Areas	30267458	32485299	4670613	55363961	14070280
	Nat. Barren Areas	5174919	5180962	683423	13301668	3111860
	Interface	42254	0	0	252929	93404
	Freshwater	871006	0	0	2972876	1077426
	Ocean	12680341	2271651	0	43420785	14535968