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Application of indicator kriging to the complementary use of bioindicators at three trophic levels

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Mercury levels in bioindicators at three trophic levels were combined using geostatistics to build an integrated environmental contamination index.

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ABSTRACT

The use of biological indicators is widespread in environmental monitoring, although it has long been recognised that each bioindicator is generally associated with a range of potential limitations and shortcomings. To circumvent this problem, this study adopted the complementary use of bioindicators representing different trophic levels and providing different type of information, in an innovative approach to integrate knowledge and to estimate the overall health state of ecosystems. The approach is illustrated using mercury contamination in primary producers (mosses), primary consumers (domestic pigeons and red-legged partridges) and top predators (Bonelli's eagles) in southern Portugal. Indicator kriging geostatistics was used to identify the areas where mercury concentration was higher than the median for each species, and to produce an index that combines mercury contamination across trophic levels. Spatial patterns of mercury contamination were consistent across species. The combined index provided a new level of information useful in incorporating measures of overall environmental contamination into pollution studies.

1. Introduction

The ubiquitous distribution of plants, lichens and animals is one of the main advantages of their use as biomonitors of environmental contamination, reducing infra-structure and manpower costs to survey large areas (Wolterbeek and Freitas, 2002). Biomonitors also allow assessment of the bioaccessibility and bioavailability of a contaminant. Mosses and lichens are probably the most used biomonitors (Nimis, 1990; Wolterbeek, 2002), due to their capacity to accumulate high concentrations of elements, especially cations, which are retained in the cell wall and membrane exchange sites, many times without affecting their intracellular physiological mechanisms (Bates, 2000). Mosses lack water impermeable cuticles, and thus obtain nutrients (and metals) through the leaf surface, not by the roots as vascular plants do (Bates, 2000). Because of these properties, terrestrial mosses are widely used as biomonitors of atmospheric deposition of trace elements, assessing environmental contamination over a wide range of spatial and temporal scales (e.g., Steinnes et al., 2003). This is the case, for instance, in the large scale survey set up at the European level and repeated regularly to assess the concentrations in mosses of a wide range of contaminants such as mercury (Rühling, 1994; Rühling and Steinnes, 1998; Buse et al., 2003). Despite their value and widespread use, mosses have some limitations to assess spatial and temporal trends in environmental contamination. In particular, these organisms do not allow any inference about the biomagnification of contaminants, which is a significant constraint for assessments at the ecosystem level.

Birds constitute another class of widely used biomonitors of pollution, with particular relevance for mercury (Furness, 1993), although it is recognised that they are limited in providing direct information on atmospheric or water contamination. For instance, mercury levels in birds may be influenced by feeding areas and

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habitats (Hoffman and Curnow, 1979), the proportion of particular prey in the diet (Lindberg and Odsjo, 1983; Palma et al., 2005), and habitat use (Jarman et al., 1996), among other factors (e.g., Gorski et al., 2003). Because of this there may be marked differences between bird and environmental contamination patterns, which can only be interpreted when there is a good knowledge of the ecology and behaviour of the species used in biomonitoring (e.g., Palma et al., 2005). Despite these problems, birds and other animals often provide useful information on environmental contamination that cannot be obtained from simpler bioindicators such as mosses. This is the case for predatory birds, which receive the contamination from different contributors at lower trophic levels (Zolfaghari et al., 2007), and may thus provide information about the integration of contamination that reaches species, such as humans, at the top of the food chain. In comparison to invertebrates, avian predators have higher potential error as indicators of environmental pollution, but only a predator can give the most realistic view about the contamination reaching the top of food webs and its effects (Chan et al., 2003).

Due to the strengths and weaknesses of each individual bioindicator, a consistent picture of environmental contamination at the ecosystem level can probably be obtained only through the complementary use of different bioindicators. Therefore, instead of using a single monitoring bioindicator, we suggest the use of an index that may integrate information from several bioindicators. This combination cannot be straightforward when different biomonitors are used, because the interpretation of the contents of a toxic element in each organism is only possible in light of the eco-physiological processes involved in that accumulation. To combine the information from several biomonitors in one index, it is thus necessary to redefine the contamination levels in order to produce unit independent variables. Such new variables could be obtained by recoding the initial values to an indicator variable, using a threshold value, and produce probability maps by indicator kriging interpolation. It is then possible to combine the maps for different species to determine an environmental indicator of metal contamination at the ecosystem level. A comparable indicator kriging approach was previously used to determine the potential health risks of arsenic contamination (Lee et al., 2007; Figueira et al., 2007) and in other environmental applications (Buttafuoco et al., 2007; Wang, 2007; Tavares et al., 2008).

The main purpose of this study was to construct an index that can integrate the level of contamination in bioindicators occupying different trophic levels. The procedure is then illustrated using a case study of mercury contamination in moss, avian primary consumers and birds of prey, as it represents three trophic levels, indicating different levels of bioaccumulation with coincidence in space representation.

2. Methodology

2.1. Study area

The study was conducted within the breeding range of Bonelli's eagle (*Aquila fasciata*) in the uplands of southwestern Alentejo and Algarve regions of southern Portugal (Fig. 1). This is a rough triangle of about 3000 km^2 linking the mountains of Cercal (341 m), Monchique (902 m) and Caldeirão (589 m) (Fig. 1). The hilly landscape is covered primarily by cork oak (*Quercus suber*) woods, dense Mediterranean scrub, and eucalyptus (*Eucalyptus globulus*) plantations, with sparse human occupation.

The main potential anthropogenic source of mercury pollution is the coal-burning power plant of Sines at the northwest end of the study area. Recently, fires might have been another significant source of mercury contamination, as large areas in the Monchique (66,000 ha) and Caldeirão (26,000 ha) mountains burned in 2003 and 2004, respectively. No additional sources of mercury pollution were identified in the study area. Mercury levels in eagles and some of their prey have been previously analysed in this region, showing that the mercury burden in eagles resulted from the joint effects of diet composition and prey contamination (Palma et al., 2005).

2.2. Study design and sample collection

This study is based on data previously reported by Palma et al. (2005) on mercury contamination in Bonelli's eagles and their main avian prey in southwest Portugal, together with novel information on mercury levels in moss samples collected within the same region. The dataset was considered adequate to illustrate the complementary use of bioindicators to assess mercury contamination at the ecosystem level, for it includes information collected over a large area on species representing trophic levels ranging from primary producers to top predators. It should be noted, however, that the moss was only used as a surrogate of bioavailable contamination at the lower trophic level, because it was not in the same food chain as the avian species. Another potential problem was the temporal mismatch in the collection of avian (1992–2001) and moss samples (2006). This was unlikely to have affected the results of the study, because the spatial patterns of mercury contamination are likely to have remained essentially constant over this study period. This is because the study was carried out in a region dominated by forest and agricultural landscape with low human occupation, where the main source of mercury contamination since the late 1970s has probably been the industrial complex of Sines (Palma et al., 2005).

The moss species selected for the study was *Scleropodium touretii* (Bird.) L. Kock, which was used in previous national surveys for this region (Sérgio et al., 1993; Figueira et al., 2002, 2004). Samples were collected on 24 sites, mainly on cork oak forest, montado ecosystem, in the first quarter of 2006. At each site, a composite sample was collected from the soil, within a 50 × 50 m area, using plastic gloves and bags to avoid handling contamination. Transport to the laboratory was done in refrigerated boxes, and the material was stored in a refrigerator (4 °C) prior to further processing to avoid sample degradation. The site selection and sampling procedures were adapted for Portugal from the European survey (Figueira et al., 2002; Buse et al., 2003). Old data was assessed for mean mercury levels in five moss samples from the same geographic area for the previous national survey performed in 2002 (Figueira et al., 2004) to check for eventual temporal variation of mercury.

Mercury levels in birds were measured in samples of feathers, because mercury in feathers is almost entirely in the form of mono-methylmercury (Thompson and Furness, 1989a,b) and reflects body mercury burdens (Thompson et al., 1990). Sampling procedures followed that described in Palma et al. (2005). Briefly, shed body feathers of adult Bonelli's eagles were collected from 21 nests and neighbouring tree perches during the breeding seasons of 1992–2001. Feathers from prey remains found at nests and perches were also collected, including domestic doves (*Columba livia*) and red-legged partridges (*Alectoris rufa*). Together with rabbits, these bird species make up the bulk of the diet of Bonelli's eagles in this region (Palma et al., 2006). Active nests were visited at least three times each breeding season, between March and July. Sampling was carried out over an extended period to obtain a sufficiently large sample, because not all nests yielded feathers of all target species every year. Mercury levels for each site and species were estimated as the corresponding average of annual mercury concentrations.

2.3. Mercury determinations

Moss samples were analysed for total mercury concentration by CV-AAS. Before digestion, the material was cleaned, and green parts were separated for digestion. The reserved material was dried overnight at 40 °C and the dry weight was determined. About 0.5 g of moss went through a wet digestion with 3 ml HNO_3 and 2 ml H_2O_2 in a microwave digestion system (Milestone MLS-1200 Mega) in closed Teflon vessels. Solutions were diluted with deionised H_2O to a final volume of 10 ml. Quality control was assessed by the inclusion of three replicates of the BCR certified reference material 482 (lichen) in the sample set; the analysis produced an average of 0.51 ± 0.02 mg/kg, with an accuracy within 6%. The detection limit (DL) was 0.027 mg/kg Hg per g, given as twice the standard deviation of triplicate analysis of blanks. Mercury concentration for mosses is given on a dry weight (d.w.) basis.

Feather samples were analysed for total mercury concentration by cold vapour atomic absorption spectroscopy (CV-AAS), using the procedure described by Palma et al. (2005). Samples were digested in a water bath at 70 °C for 6 h by the addition of concentrated H_2SO_4. After this period 5% $KMnO_4$ was added and the solution was kept at 70 °C for a further 2 h. Excess $KMnO_4$ was reduced with 20% $NH_2OH{\cdot}HCl$. All reagents used throughout the work were of analytical grade. The glassware was previously decontaminated by immersion in a HNO_3 1:5 solution and then washed with deionised water. Reproducibility was checked by performing successive measurements with the same sample. Relative standard deviations in the range 3–5% were found. The accuracy of the method was within 10% and was monitored by analysing reference materials: NIES-5 human hair from NIES-Japan (certified value 4.4 ± 0.4 mg/kg) using samples between 0.010 and 0.030 g d.w. (average = 4.1 mg/kg, std = 0.03, c.v. = 7.95%, n = 7), and CRM 463 tuna fish from BCR-Belgium IAEA (certified value 2.85 ± 0.17 mg/kg) using samples between 0.003 and 0.018 g d.w. (average = 2.66 mg/kg, std = 0.02, c.v. = 0.8%, n = 5). The detection limit (DL) of 0.01 mg/kg Hg per g digested sample was given as twice the standard deviation of triplicate analysis of blanks (Saltzman et al., 1983); blank values were above the base line of the equipment. Mercury concentration for birds is given on a fresh weight (f.w.) basis.