Structural alterations as biomarker in the shrew *Crocidura russula* exposed to chronic pollution

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This study assesses the effects of environmental pollution in a protected site using a histopathological evaluation with light microscopy. Historically impacted by anthropogenic activities, the nature reserve of Doñana (SW Spain) was affected by an unprecedented spillage of mud and acidic water from the Aznalcóllar pyrite mine in April 1998. Although several studies have addressed the influence of this spill on soils, water, and biota, there is little information on mammals, especially carnivorous species. We examined several biomarkers including quantitative and qualitative evaluation of structural alterations to approach at the physiological effects of pollution in specimens of the greater white-toothed shrew, *Crocidura russula*, inhabiting the protected area affected by the mine spillage. We also correlate these measures with the concentrations of Tl, Fe, Mg, Pb, Hg, Cd, Zn, Cu, Mn, Mo, Co, and Cr. Specimens from the polluted site showed an increase in non-essential metals (Tl, Pb, Cd, Hg), and morphometric, genotoxic and structural alterations. Shrews from the impacted area also had hepatic alterations, namely increased liver-body ratio, focal necrosis, and signs of apoptosis in hepatocytes (**Figure 1**). These morphometric and structural findings point out in the physiological effects of metal pollution in wild populations. Due to the relevance of small mammals in the diet of endangered species such as carnivorous birds and mammals, the findings of our study are of practical use for the management of the Doñana wildlife reserve and other protected Mediterranean wetlands.

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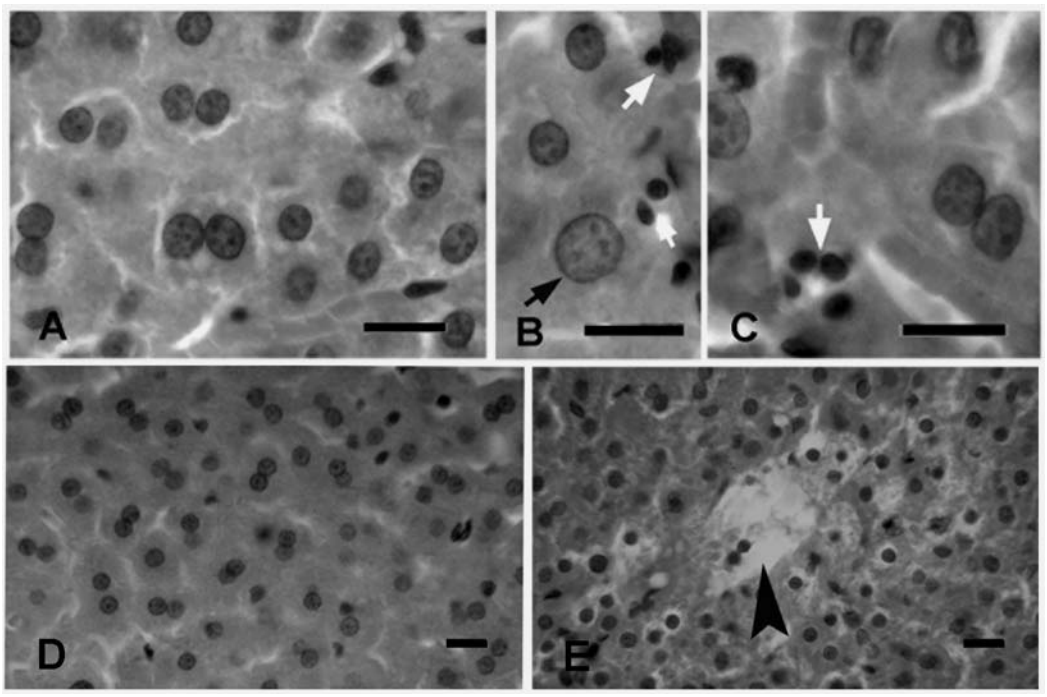

Figure 1. Hepatic sections showing normal and altered tissue in *C. russula*. (A,D) Healthy livers from shrews collected at the reference site; (B,C,E) Livers from shrews collected at the polluted site: Observe the enlarged nuclei (black arrow) (B), the apoptotic figure (white arrow) (B,C), and the foci of cell necrosis (black arrow) (E). Haematoxilin and eosin stains. (Scale bars: A,D,E = 50 μm; B,C = 20 μm).

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