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The Italian wall lizard (*Podarcis sicula*) as a bioindicator of oil field activity

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ABSTRACT

The aim of this study was to develop and to validate a methodology based on biomarker responses and residue analysis on the terrestrial lizard *Podarcis sicula* to assess the ecotoxicological effects associated with on-shore oil extraction. The oil treatment plant investigated is located in Val d'Agri (southern Italy). Italian wall lizards were sampled on four stations along a transect determined on the basis of prevailing winds downwind of the oil plant. Cytochrome P450 1A1 activities (EROD and BPMO), AChE activity, PAH bile metabolites and contaminant levels (PAHs and trace elements) were measured. Major results in the evaluation of toxicological impact of oil field activity in the Italian wall lizards were obtained for Cd, Hg, total and carcinogenic PAH levels, and PAH metabolites in bile. Results obtained validate, for the first time, *P. sicula* as a terrestrial bioindicator for the assessment of the toxicological impact of on-shore extraction activity.

1. Introduction

With the exception of a few species, the use of reptiles as bioindicators of environmental contamination is a quite recent proposal. Some of the many reasons for this are the difficulty of sampling sufficiently large numbers, an ancient fear, lack of economic importance of reptiles and the difficulty of acclimatising them in the laboratory (Loumbourdis, 1997). However, their persistence in a variety of habitats, wide geographic distribution, longevity and, in many cases, site fidelity, actually make them very suitable bioindicators of contamination (Crain and Guillette, 1998). Moreover certain groups are near to the top of the food chain (crocodiles, snakes) and they bioaccumulate and biomagnify contaminants to level similar to birds and mammals (Crain and Guillette, 1998). A review about the accumulation of environmental inorganic and organic contaminants and radionuclides on lizards was performed by Campbell and Campbell (2000, 2002). In these studies the importance of lizards as bioindicators in ecotoxicological risk assessment was emphasized, particularly in relation to pesticides and polychlorinated biphenyls (PCBs). The first author to propose the use of lizards as bioindicators of pesticide contamination was Lambert in 1987. Al-Hashem et al. (2007) analysed polycyclic aromatic hydrocarbons (PAHs) in the desert lizard (*Acanthodactylus scutellatus*) to monitor the effects of oil pollution at Kuwait's greater Al-Burgan oil field, 12 years after the Kuwait oil spill catastrophe. The range of total PAHs was 26.1–301 ng/g dry weight (d.w.) and the concentrations increased progressively along an expected contamination gradient. Few studies were conducted in lizards, and generally in reptiles, about toxic effects of environmental pollutants. The induction of cytochromes P450 by barbiturate phenobarbital (PB) is a general phenomenon in mammals and birds, but suggestive data showed for the first time cytochromes P450 induction in the lizard *Agama* lizard treated with PB (Ertl and Winston, 1998). In several papers, the lizard *Gallotia galloti* was used for the assessment of the toxicological impact of organophosphorus (OP) and carbamate (CB) insecticides using butyrylcholinesterase (BChE) and carboxylesterase (CbE) activities in serum as nondestructive biomarker (Fossi et al., 1995; Sanchez et al., 1997; Sanchez-Hernandez and Sanchez, 2002; Sanchez-Hernandez, 2003; Sanchez-Hernandez et al., 2004). The results showed that serum BChE activity was inhibited by both OP and CB pesticides and CBs appeared to be the pesticides most responsible for BChE inhibition. Moreover BChE activity of *G. galloti* was a very sensitive biomarker of pesticide exposure, with a slow recovery rate (over weeks) after acute OP exposure. In the lizard *Podarcis sicula* exposure to sub-lethal concentrations of the fungicide methyl thiophanate (TM) leads to hepatocellular morphological changes and glycogen depletion, apoptosis, as well as probable peroxisomal proliferation attested by the increase of acyl-CoA oxidase (AOX) (Buono et al., 2007). On the basis of these results the authors proposed peroxisomal proliferation and AOX increase as new biomarkers to evaluate pollution by organic compound in terrestrial environments. Moreover lizards are particularly good models for studying endocrine altering compounds (Brasfield et al., 2002; Chieffi et al., 2002). Vitellogenin was induced in male western fence lizards (*Sceloporus occidentalis*) by 17β-ethinylestradiol (EE_2)

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(Brasfield et al., 2002) and spermatogonial proliferation was induced in the male lizards *P. sicula* by 17β-estradiol (E) (Chieffi et al., 2002), both estrogenic compounds.

In this study, we propose the Italian wall lizard *P. sicula* as terrestrial bioindicator for ecotoxicological assessment of the impact of an on-shore oil treatment plant in the southern Italy. This species was selected due to its wide distribution in mainland and islands of Italy, its habitat (fields, walls, areas up to 1800 m), limited mobility and insect diet. Indeed, lizards are an important link between invertebrates and higher predators, and whole-body residue loads provide an indication of levels entering the food chains of wildlife. Moreover, lizards, being soil surface animals, can be affected by contamination from pesticides and chemical substances present in the soil or contaminants that reach the soil surface through fall out. Lizards, as other reptiles, are ectothermic, with low metabolic rate. Additionally, they have relatively simple enzyme systems and a poor ability to detoxify pesticides absorbed, inhaled or ingested (Walker and Ronis, 1989). Lizards are especially valuable as bioindicators during dry and hot seasons (Lambert, 1994, 2004) because they do not tolerate low temperatures (Burke et al., 2002).

Two large onshore oil fields in the Val d'Agri area (Basilicata Region, southern Italy), about 30 km south of Potenza, are exploited under two concessions (Grumento Nova and Volturino), in which ENI and Enterprise Oil hold shares. Commercial production from Val d'Agri began in April 1996. In the study period (2000–2001), production was transported from the Monte Alpi oil center (Viggiano) to the AGIP Petroli refinery at Taranto, and associated gas production was fed into the national gas network. ENI and Enterprise Oil have entered into a series of agreements with the Basilicata regional authorities to provide environmental compensation, complementary to the overall Environmental Management Plan prepared by ENI, establishing an integrated environmental monitoring network. The BIOAGRI pilot project belongs to this framework, with the aim of developing and validating a new method, based on biomarkers and residue analysis in bioindicator organisms, to evaluate the ecotoxicological status of the area surrounding the Monte Alpi oil center. The toxicological impact of the plant on freshwater ecosystems (fish experiment), on terrestrial ecosystem (lizard experiment) and related to the air emissions (rats experiment) was evaluated by a "multi-trial biomarker approach" (Ferraro et al., 1999; 2000). The lizard *P. sicula* was proposed as a terrestrial bioindicator for the assessment of the plant impact, testing a large suite of biomarkers in different biological materials and estimating levels of major oil-storage-related contaminants (PAHs and trace elements) in lizard bodies. In fact crude oils are complex mixtures which vary widely in composition. Concentrations of PAHs in crude oil are several parts per million (almost invariably < 1 mg/kg; only phenanthrene, anthracene and fluoranthene are sometimes found at > 10 mg/kg) (Herlan, 1982) and, by their stable structures, PAHs persist in the environment long after other oil field product constituents have degraded (Sugiura et al., 1997). The United States Environment Protection Agency (EPA) (Francis, 1994) and the World Health Organisation (WHO, 1970) have identified 16 PAHs as priority pollutants, classified into two major groups: carcinogenic and non-carcinogenic PAHs. The first group include seven PAHs: benzo(a) anthracene, chrysene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(k) fluoranthene, dibenz(a,h)anthracene, and indeno(1,2,3-cd)pyrene, all

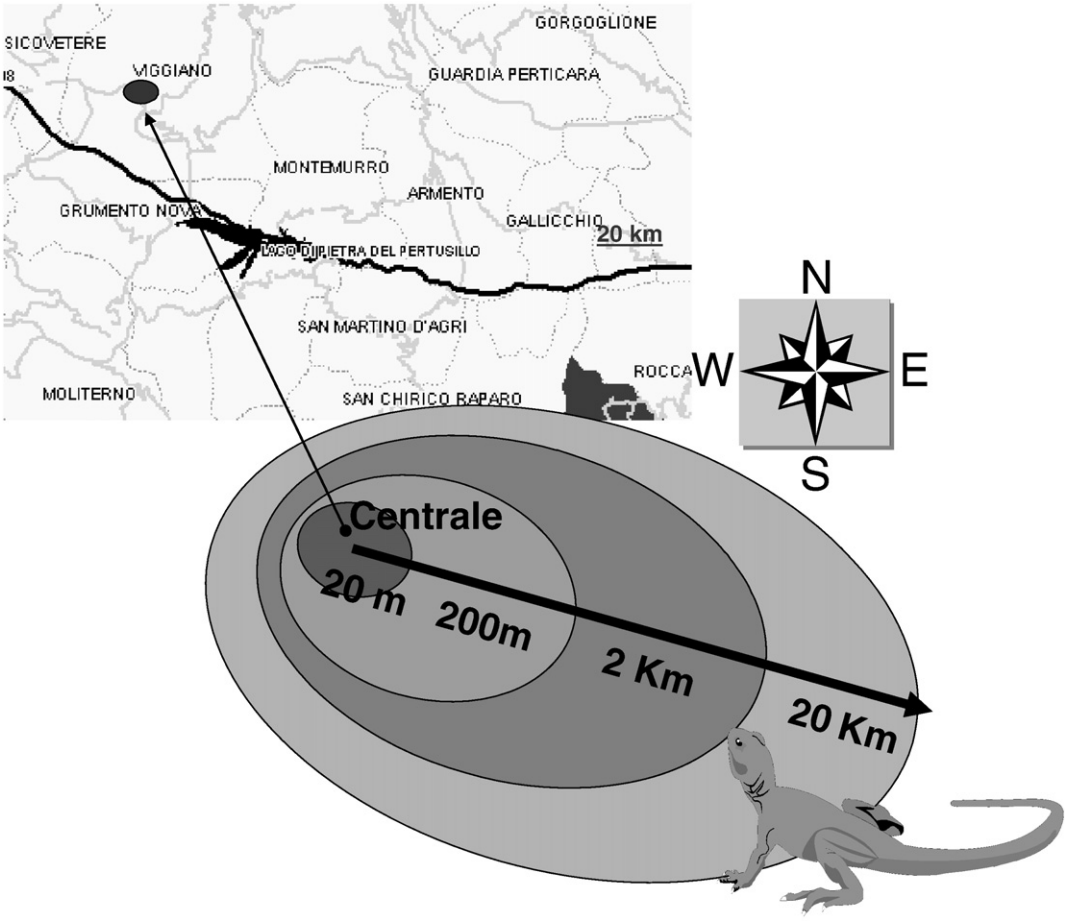

Fig. 1. Sampling plan with terrestrial reptile bioindicator. Specimens of the lizard *Podarcis sicula* were captured in June 2000 in four sampling stations at distances of 20 m, 200 m, 2 km and 20 km from the Monte Alpi oil center in the direction of prevailing winds.