Ecotoxicology (2012) 21:2103–2112
DOI 10.1007/s10646-012-0978-0

Pollution biomarkers in the spiny lizard (*Sceloporus* spp.) from two suburban populations of Monterrey, Mexico

Carlos Aguilera · Pamela González del Pliego · Roberto Mendoza Alfaro · David Lazcano · Julio Cruz

Accepted: 14 July 2012 / Published online: 8 August 2012

Abstract Environmental pollution may severely impact reptile species in urbanized areas. The magnitude of the impact is analyzed in the present study using lizard tail tips for the quantitative evaluation of enzymatic biomarkers of pollution. Spiny lizards (*Sceloporus serrifer* and *S. torquatus*) were collected from two suburban localities in the Monterrey metropolitan area, Mexico: Chipinque Ecological Park, a natural protected area, and El Carmen Industrial Park (IP), a highly polluted site. Different enzymes were used as biomarkers including: acetylcholinesterase (AChE), butyrylcholinesterase (BChE), carboxylesterase (CaE), alkaline phosphatase (ALP), acid phosphatase (ACP), superoxide dismutase (SOD) and glutathione S-transferase (GST). The levels of AChE, BChE and ACP activity were not significantly different between localities. AChE and BChE, commonly used as biomarkers of neurotoxic polluting agents (e.g. organophosphate pesticides) do not appear to be affecting the populations from the study locations. In contrast, the levels of CaE, GST, ALP and SOD were significantly different between the localities. These biomarkers are regularly associated with oxidative stress and processes of detoxification, and generally indicate pollution caused by heavy metals or hydrocarbons, which are common in industrial sites. The data resulting from the analysis of these biomarkers indicate that these polluting agents are affecting the populations of *Sceloporus* in IP. The present work validates the possibility of conducting additional ecotoxicological studies using biomarkers in combination with a nondestructive sampling technique in species of spiny lizards that are abundant in many North America areas.

Keywords Reptiles · Lizards · Biomarkers · Pollution · *Sceloporus*

C. Aguilera · R. M. Alfaro (✉) · J. Cruz
Laboratorio de Ecofisiología, Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León, UANL, Apartado Postal F-96, 66450 San Nicolás de los Garza, NL, Mexico
e-mail: mendozar787@yahoo.com

P. G. del Pliego · D. Lazcano
Laboratorio de Herpetología, FCB, Universidad Autónoma de Nuevo León, UANL, San Nicolás de los Garza, Mexico

Introduction

There is a generalized consensus concerning the ecological importance of herpetofauna and its high sensitivity to the environmental pollution, (Campbell and Campbell 2002). However, reptiles continue to be under-utilized as sentinel species in ecotoxicological studies, in spite of recommendations for their use (Schmidt 2003). Among reptiles, lizards are particularly suitable as pollution biomonitors, as a result of their presence in a variety of habitats, wide geographic distribution, longevity, and in many cases site fidelity (Lambert 2005). Furthermore, lizards are especially useful during dry seasons or in arid regions (Sciarrillo et al. 2008). Lizards proposed as sentinel species include: *Gallotia galloti* (Fossi et al. 1995; Sanchez-Hernandez and Moreno 2002), *Acanthodactylus dumerili* (Peveling and Demba 2003) and *Agama* sp. (Adeyemi and Adedeji 2006) in Africa; *Calotes versicolor* (Khan and Fatima 2002; Khan 2003) in Asia; and *Podarcis sicula* in Europe (Trinchella et al. 2006; Marsili et al. 2009; Favorito et al. 2010). While recently in North America, different species of spiny lizards (*Sceloporus* sp.) have been proposed as models to be used in ecotoxicological studies and environmental risk assessments, because they are small, reach maturation in a short time, and are able to reproduce under laboratory conditions (Talent et al. 2002; Unrine et al. 2006; Holem et al. 2006,

2008; DuRant et al. 2007; Salice et al. 2009; McFarland et al. 2011).

Recently, it has been documented that the exposure of lizards and snakes to neurotoxins provokes damages with repercussions on their locomotive or swimming performance and general fitness (Hopkins et al. 2005; Hopkins and Winne 2006; Holem et al. 2006, 2008; DuRant et al. 2007). It has also been confirmed that the processes of accumulation of pesticides, metal and polycyclic aromatic hydrocarbons is related to reproduction alterations and other effects on lizards (Márquez-Ferrando et al. 2009; Favorito et al. 2010). However, the studies based on the use of biochemical biomarkers to evaluate the effect of polluting agents in lizards are scarce, even though these have shown to have a great sensitivity and utility in other organisms (Van der Oost et al. 2003). Cholinesterases enzymes (ChE) used to monitor their inhibiting compounds, chiefly organophosphates pesticides, are among the most broadly employed biomarkers in lizards (Sanchez-Hernandez and Walker 2000; Sanchez-Hernandez and Moreno 2002; Schmidt 2003). Glutamate oxaloaceate transaminase and glutamate pyruvate transaminase activities have been shown to decrease by the exposure to pyrethroid and organophosphate pesticides in the lizard *Calotes versicolor* (Khan and Fatima 2002). Enzymatic activities of cytochrome P450 and acetylcholinesterase (AChE) were used to assess the toxicological impact of oil extraction activity in *Podarcis sicula* (Marsili et al. 2009). Alterations in alkaline phosphatase (ALP) activity, albumin, total protein, and, calcium blood levels were shown in *S. occidentalis* after exposure to TNT (McFarland et al. 2011).

An advantage to use reptiles as sentinel organisms is the availability of several nondestructive techniques, particularly, blood, skin and tail analyses, which have a great potential for use in the field as well as in the laboratory (Hopkins et al. 2001; Jones et al. 2005; Burger et al. 2005; Hare et al. 2005). Within this context, the present work was aimed at determining the levels of enzymatic activities in wild populations of the spiny lizards (*Sceloporus serrifer* and *S. torquatus*) (Stebbins 2003) from two different locations near the metropolitan area of Monterrey, Mexico. The research was based on the use of tail tips, which easily regenerate, as a nondestructive technique.

Method

Study area and animal sampling

The study areas are located in the Monterrey city limits. The Chipinque Ecological Park (EP) is a natural protected area, located in the southwest of the metropolitan area. This ecological park has an area of 1,825 hectares and an altitude of 800–2,200 masl (Fig. 1). The second site is the El Carmen Industrial Park, which is a suburban highly polluted area, with established metallurgical smelting industries located to the northwest of the metropolitan area, with an area of 120 hectares and an average altitude of 500 masl. The climate in this zone is semiarid, with annual temperatures averaging 18–22 °C, winter minimum temperatures of 0–4 °C, and summer maximum temperatures of 22–35 °C. The annual average precipitation oscillates between 300 and 500 mm.

Lizards were collected during the months of May and June of 2009. In EP established pathways were selected to search for lizards between the vegetation and the substrate and these were used as transects with lengths between 0.2 and 2.7 km. In IP transects were established randomly between the streets of the park, where the lizards were located in peripheral walls of existing industries or factories. The lizards were captured manually or by means of nylon lizard noose and the following data were recorded for each captured lizard: species, gender, weight, snout-vent length (SVL), tail length (TL), tail length of regenerated tails (TL-RT) and tail width at base (TW). Main physical characteristics were recorded for the site (hour, coordinates, altitude, vegetation, substrate, environmental and substrate temperatures, and relative humidity). After the lizards were captured, 3–5 cm of the distal portion of the tail were removed with surgical scissors. The tail samples were labeled and individually stored in ice at 0 °C for their transport to the laboratory that same day, where they were frozen at −70 °C until they were processed. The tail cut was disinfected with iodopovidone and the organisms were released in situ.

Sample preparation

The tail samples were individually weighed in a digital analytical balance with 0.01 mg precision, the tail was finely cut in pieces using scissors and a bistoury in a petri dish sitting on ice. The prepared tissues were homogenized at 4 °C using a Wheaton-glass homogenizer (Glas-Col^{TM}) at 333 rpm during 4 min in tris–HCl 50 mM (pH 7.1) buffer to a proportion of 1:10 (w/v). The homogenized material was centrifuged at 15,300×*g* at 4 °C for 30 min. The supernatant was separated from the superior lipidic layer and from the precipitate. The supernatant was stored in 0.1 mL aliquots at −70 °C to be later used as an enzymatic extract. Total soluble protein concentrations in the extracts were determined by the Bradford (1976) method with bovine albumin serum (BSA) as a standard.

Esterases assays

The methodology described by Ellman et al. (1961), was followed for esterases determination. The technique modified

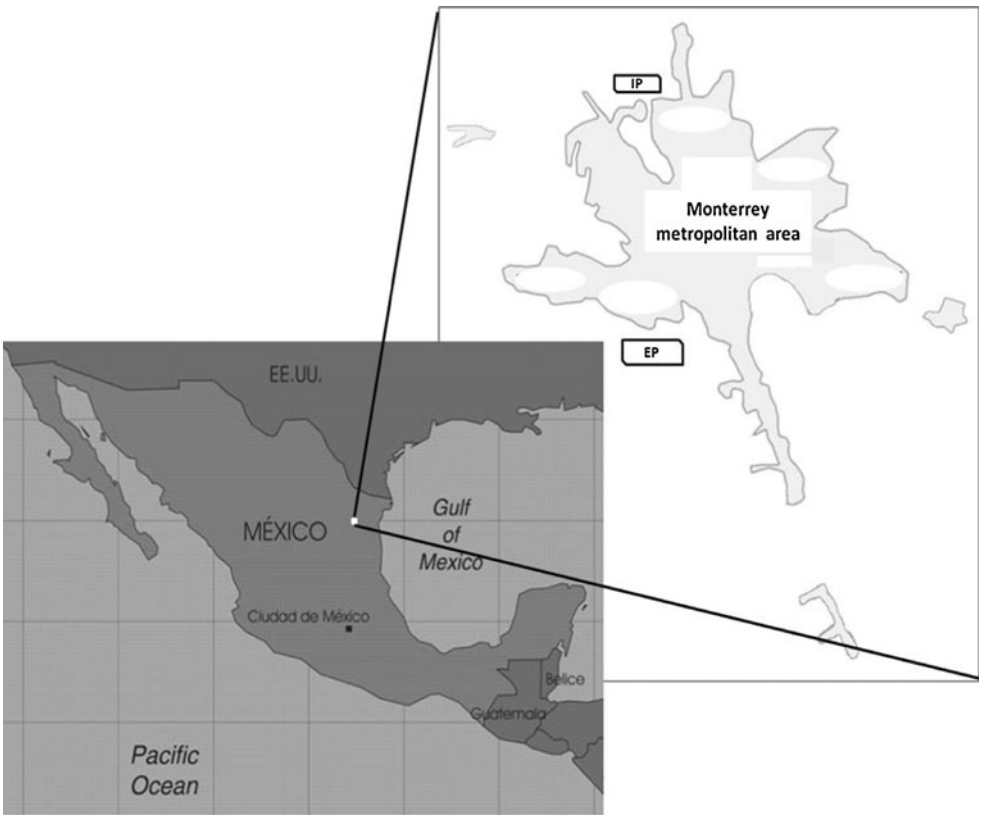

Fig. 1 Sampling sites where individuals of spiny lizards (*Sceloporus* spp.) were collected. The map shows the surroundings of the metropolitan area of Monterrey, Nuevo León, Mexico. *EP* Chipinque Ecological Park, *IP* El Carmen Industrial Park

by Huang et al. (1997) for its application in microplates was used. For AChE and butyrylcholinesterase (BChE) the reaction mixture consisted of 280 µL of 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) in buffer PBS 0.1 M (pH 7.8) and 10 µL of enzymatic extract. The reaction was initiated adding 10 µL of acetylthiocholine chloride (0.015 M) for the AChE activity or butyrylthiocholine chloride (0.015 M) for the BChE activity. For carboxylesterases (CaE) 200 µL of buffer Tris–HCl 50 mM (pH 7.1), 10 µL of enzymatic extract and 100 µL of *p*-nitrophenyl acetate (2 mM) like substrate were added to initiate the reaction. Absorbance was immediately registered at 405 nm in intervals of 120 s up to 10 min in a microplate reader (TECAN, Sunrise^{TM}). For each sample three analytical replications were conducted. In the case of the control the sample was replaced with buffer. The linearity of the reaction was verified and the enzymatic activity was expressed as the increase of absorbance per minute by milligram of protein in the extracts (ΔAbs/min/mg protein).

Phosphatases assays

Alkaline and acid phosphatase (ALP and ACP) activities were measured using *p*-nitrophenyl phosphate as substrate (Mazorra et al. 2002). The reaction was performed using 200 µL of buffer diethanolamine (1.0 M) with 50 mM $MgCl_2$ (pH 9.8) for ALP or buffer 0.1 M sodium acetate-HCl (pH 4.8) for ACP, then 10 µL of the enzymatic extract and 10 µL of the substrate were added at a final concentration of 0.4 mM. Absorbance was immediately registered at 405 nm in the same conditions as the esterases measurement and enzymatic activity was expressed as ΔAbs/min/mg protein.

Glutathione S-transferase (GST) assay

The GST activity was analyzed according to Wilce and Parker (1994). The reaction mixture was carried out in 1.0 mL quartz cells and consisted in 970 µL of Dulbecco's buffer (pH 7.2), 10 µL of reduced L-glutathione (200 mM) and 10 µL of the enzymatic extract. The reaction was initiated by adding 10 µL of 1-chlorine-2,4-dinitrobenzeno (CDNB) 100 mM. Absorbance was immediately registered at 340 nm every 30 s during a period of 5 min in SPECTRONIC spectrophotometer, Genesys 2. GST activity was expressed as µmol/mL/min/mg protein using for CDNB a molar extinction coefficient of 9.6/Mmol/cm.

Superoxide dismutase (SOD) assay

A kit (CAYMAN 706002) was used to determine the SOD activity. The reaction mixture was carried out in microplates, and consisted of 200 µL of radicals detector (tetrazolium salt), a 50 mM Tris–HCl buffer (pH 8.0) with diethylenetriaminepentaacetic acid (0.1 mM) and hypoxanthine

(0.1 mM), and 10 μL of enzymatic extract or a SOD standard. The reaction was initiated by adding 20 μL of xanthine oxidase in 50 mM Tris–HCl buffer (pH 8.0), catalyzing the liberation of superoxide radicals, which were caught by the tetrazolium salt and quantified at 450 nm. The concentration of the radicals was inversely proportional to the SOD activity in the samples. In order to quantify the activity, a SOD standard curve was used and the concentration in the samples was expressed in U/mL, defined as the amount of SOD necessary to exhibit 50 % of dismutation of the superoxide radicals.

Statistical analysis

The data were grouped in blocks considering factors such as: locality (EP or IP); species [*S. serrifer* (S) or *S. torquatus* (T)]; and gender [female (F) or male (M)]. The groups were identified by combining two or three of these factors, for example EPSF refers to a female of *S. serrifer* from the Ecological Park. In the case of the data corresponding to the morphometric and enzymatic activities, statistical analyses were performed using SPSS 13.0 software (SPSS Inc., Chicago IL USA). Differences between blocks were determined by means of a one-way analysis of variance (ANOVA). Data were tested using the Levene's test for homogeneity of variances and a post hoc analysis of Tukey (Levene $p > 0.05$) or Games-Howell (Levene $p < 0.05$) for multiple range test.

Results

Environmental parameters

Twenty individuals of *S. serrifer* were captured at the EP, nine females (EPSF), ten males (EPSM) and one undetermined juvenile. In contrast, only seven individuals of *S. torquatus* were collected, two females (EPTF) and five males (EPTM). From IP 27 individuals of *S. serrifer* were collected, 17 females (IPSF) and ten males (IPSM) and no *S. torquatus* were found at this locality. Overall, the gender of the individuals was equal with a slight majority of females (54 %). The temperature at both sites varied between 20 and 45 °C. Individuals were found most commonly between 26 and 29.9 °C, and this range was also the most usual for the micro-substrate where individuals were located. An important proportion (50 %) was captured at an altitude ranging from 520 to 550 masl and the rest between 550 and 1,300 masl. In EP the oak forest was the preferred vegetation type for both species (75 %), whereas at IP the individuals were found mostly at the submontano scrub. More than 50 % of the individuals were captured in habitats where the relative humidity prevailed between 25 and 40 % and the time when the individuals were most abundant was between 14:00 and 15:00 h on sunny days. The substrate most commonly used by *S. serrifer* individuals was construction walls (68 %) followed by rocks (30 %). Whereas *S. torquatus* were found most commonly on trees (43 %) and rocks (43 %).

Morphometric features

Regarding the morphometric variables considered, since few differences ($p < 0.05$) were obtained when the gender was considered in blocks, with the exception of SVL between EPSF and EPSM, males and females were pooled to increase the sample size and have a more robust statistical analysis. Significant differences ($F = 3.851$, $df = 50$, $p = 0.028$) were found for body weight of *S. serrifer* between localities (EPS and IPS), with higher values for those individuals inhabiting the Industrial Park (Fig. 2). Also significant differences were found concerning the snout-ventral length ($F = 5.242$, $df = 49$, $p = 0.009$) and tail length ($F = 7.549$, $df = 25$, $p = 0.003$) between individuals of *S. torquatus* from the Ecological Park (EPT) and those of *S. serrifer* from the Industrial Park (IPS). No significant differences were registered for TL-RT and TW.

Enzymatic biomarkers

In the same way as in the case of morphological variables, few differences ($p < 0.05$) were obtained when the gender was considered in blocks (ACP between EPSF and EPSM; GST between EPTF and EPTM), so genders were pooled to have a more robust statistical analysis. No significant differences were found for the AChE activity in any of the groups (Fig. 3). For the BChE activity significant differences ($F = 4.091$, $df = 149$, $p = 0.19$) were determined as a result of the lower activity in EPS compared to IPS. Whereas for the CaE significant differences ($F = 186.759$, $df = 152$, $p < 0.001$) were determined by the higher activity levels found in individuals from Industrial Park (IPS) compared to both groups from the Ecological Park (EPS and EPT).

Significant differences were also found for the ALP activity ($F = 33.674$, $df = 152$, $p < 0.001$), as a consequence of the lower activity displayed by the individuals from the Industrial Park (IPS) compared to individuals from the Ecological Park, and also between species of the former due to the lower activity in *S. serrifer* (EPS) compared to that of *S. torquatus* (EPT). No significant differences were found for the ACP activity. Significant differences were found for the SOD activity ($F = 14.273$, $df = 101$, $p < 0.001$). These differences could be attributed to a higher activity in *S. serrifer* from the Industrial Park (IPS) compared to the activity displayed by individuals of the same species from the Ecological Park (EPS).

Significant differences were found for the GST activity ($F = 26.78$, $df = 99$, $p < 0.001$, $n = 100$). In this case a

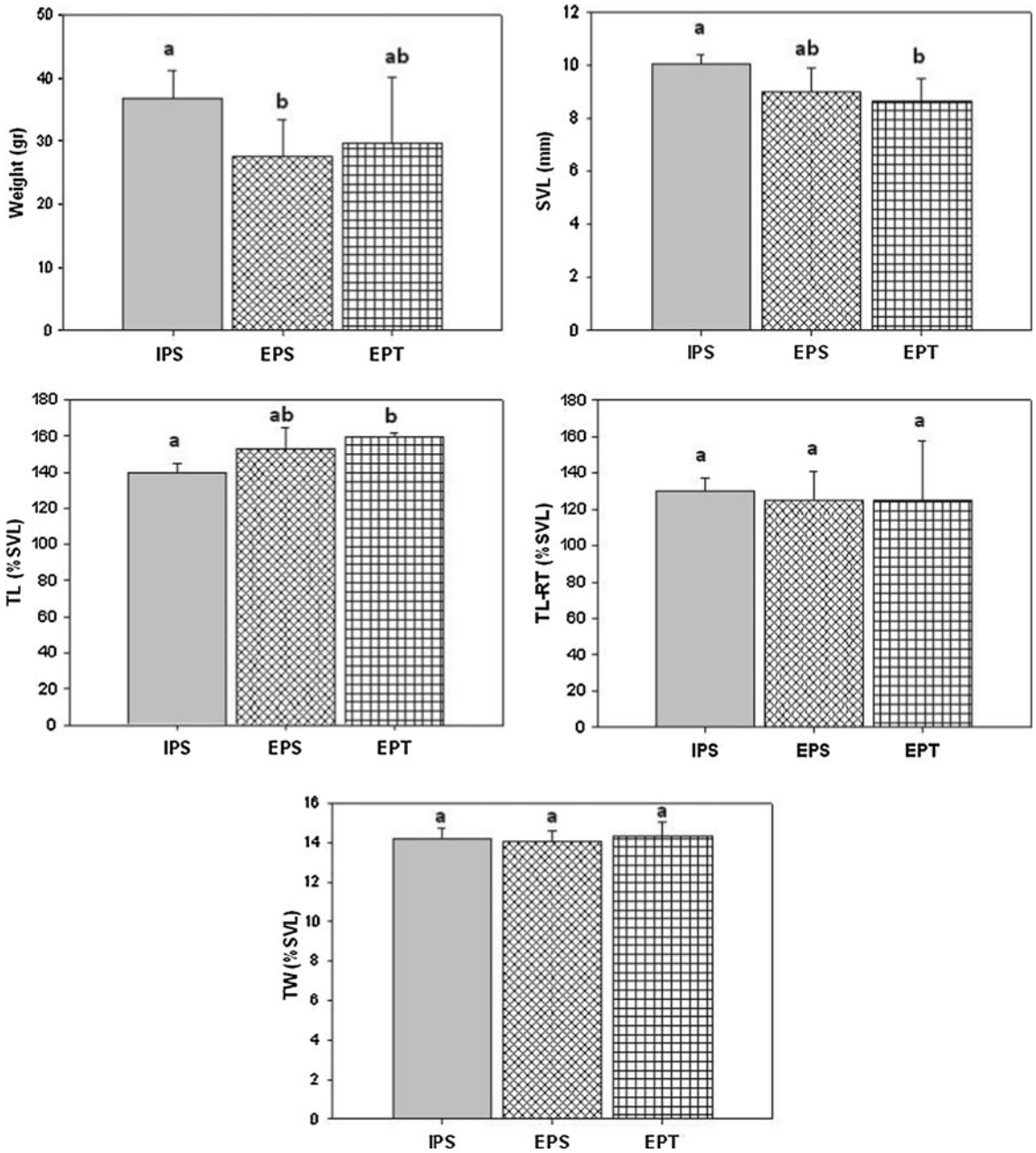

Fig. 2 Morphological variables determined in individuals of spiny lizards near the metropolitan area of Monterrey, Mexico. *IPS S. serrifer* from the Industrial Park, *EPS S. serrifer* from the Ecological Park, *EPT S. torquatus* from the Ecological Park, *BW* body weight (gr), *SVL* snout vent length (mm), *TL* tail length, *TL-RT* tail length of regenerated tails, *TW* tail width. Values are mean ± standard deviation. *Bars* with the same superscript are not significantly different ($p > 0.05$)

marked difference in activity between localities was registered, independently of the species and gender. The organisms from Industrial Park displayed a higher GST activity, compared with individuals from Ecological Park.

Discussion

In the present study the species were found to be diurnally active and a higher number of individuals were captured between 26 and 30 °C. This corresponds to the reported preferred temperature for *S. serrifer* (24–38 °C), and that of *S. torquatus* which has been reported to be active on sunny winter days (Contreras-Lozano et al. 2007). The range of temperature registered in this study may be considered narrow and therefore nondetermining in the enzyme activity analysis. This is supported by the absence of statistical correlation between the environmental temperature and the enzymatic activity (Table 1). In relation to this, some studies indicate that reptiles show a combination of

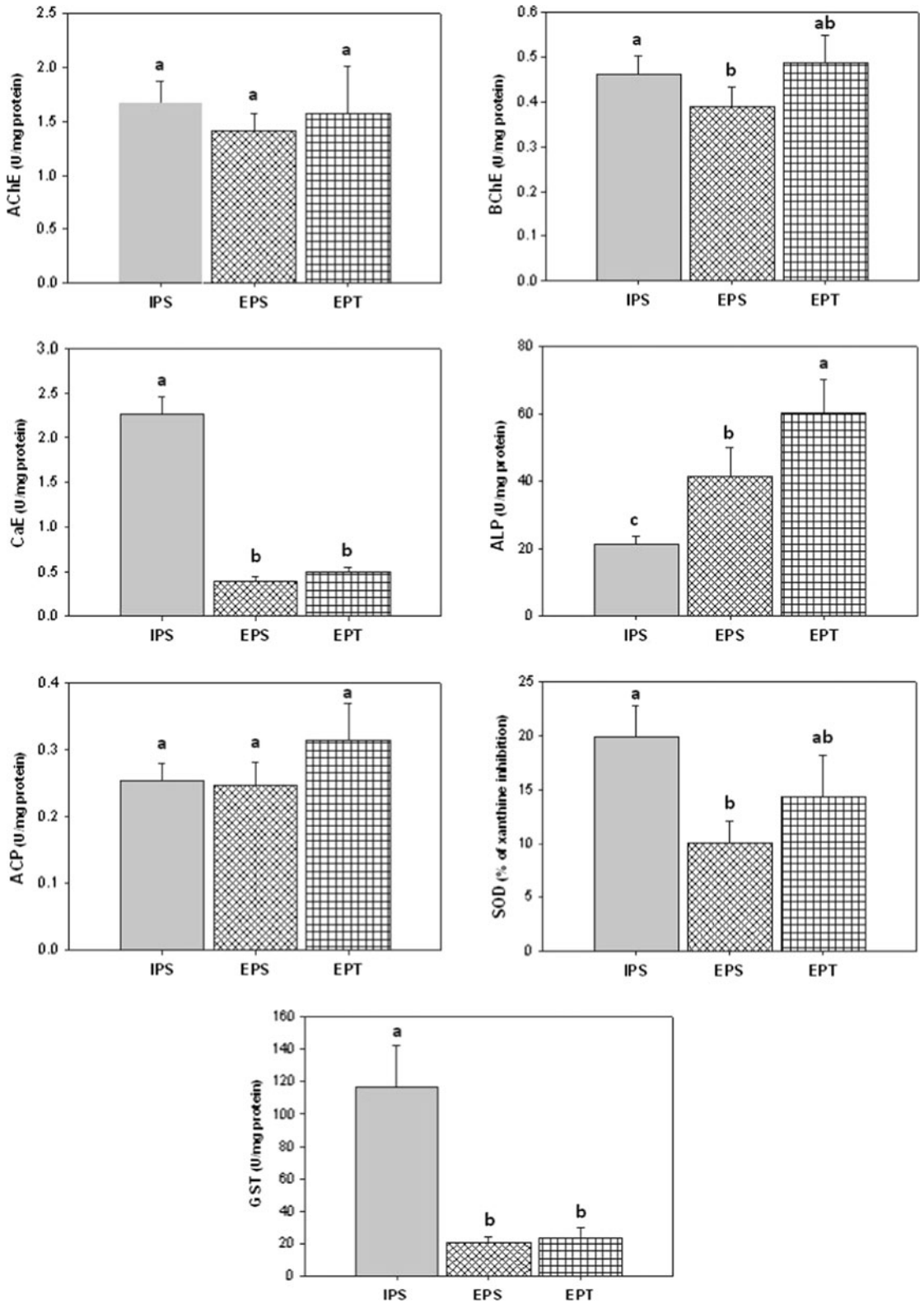

Fig. 3 Enzymatic activity (U/mg protein) of enzymatic biomarkers in tail samples of individuals of spiny lizard collected in the surroundings of the metropolitan area of Monterrey, Mexico. *IPS S. serrifer* from the Industrial Park, *EPS S. serrifer* from the Ecological Park, *EPT S. torquatus* from the Ecological Park, *AChE* acetylcholinesterase, *BChE* butyrylcholinesterase, *CaE* carboxylesterase, *ALP* alkaline phosphatase, *ACP* acid phosphatase, *SOD* superoxide dismutase, *GST* glutathione S-transferase. Values are mean ± standard deviation. *Bars* with the same superscript are not significantly different ($p > 0.05$)

Table 1 Correlation coefficient (r^2) between the environmental temperature and the enzymatic activity of the biomarkers analysed in individual of spiny lizard collected in the surroundings of the metropolitan area of Monterrey, Mexico

	EP	IP
AChE	0.173	0.027
BChE	0.26	0.016
CaE	0.32	0.048
ACP	0.005	0.002
ALP	0.007	0.004
SOD	0.049	0.003
GST	0.011	0.002

EP Chipinque Ecological Park, *IP* El Carmen Industrial Park

thermoregulatory-behavior and biochemical acclimatization in response to changes in environmental conditions. For example the citrate synthase activity in *Chelodina longicollis* did not respond to seasonal changes (Seebacher et al. 2004), in the same sense neonates of *Chrysemys picta* exposed at different temperatures did not show differences in the antioxidative capacity of the plasma (Baker et al. 2007). Also when *Lacerta vivipara* was submitted to temperatures below the freezing point (−2.5 °C), it did not show any changes in SOD and glutathione peroxidase enzymatic activity (Voituron et al. 2005). Finally, in a study where lizards adapted to low nocturnal temperatures (5 °C) were compared to those adapted to high diurnal temperatures (35 °C), no differences in the lactate dehydrogenase enzymatic activity were found (Hare et al. 2005).

Most of the individuals were collected in a narrow margin of altitude (525–530 msnm), considering the broad range of altitude covered in this study (520–1,310 msnm). The reported altitudinal distribution gradient for *Sceloporus* species is quite large, starting below sea level and extending up to 4,110 masl (Stebbins 2003). Also in the northeast of Mexico *S. serrifer* and *S. torquatus* has been reported to exist between 270 and 2,800 masl (Contreras-Lozano et al. 2007). Therefore, the distributional altitudinal gradient of these individuals does not seem to be crucial in determining the enzymatic activity of the biomarkers. As an example, Hofer et al. (2005) did not find any alterations of the oxidative stress among populations of tadpoles of *Rana temporaria* in an altitude gradient range between 1,465 and 2,150 masl.

Concerning the relative humidity, 69 % of the animals were collected in habitats within a humidity range between 30 and 49.9 %. In this case, it does not seem to exist a physiological impact of this factor, due to the fact that these organisms are able to regulate their activity and water loss, and thus can withstand long periods of drought (Nicholson et al. 2005).

Similar results of AChE and BChE activity between individuals of both sites in this present research allowed us to assume that there is no differential exposition to organophosphate pesticides, carbamates or other neurotoxic polluting agents. The inhibition of ChEs is widely used as biomarker in instances of exposure to organophosphate and/or carbamates pesticides in mammals, birds, fish and invertebrates (Thompson and Walker 1994; Thompson 1999). It has been observed that the sensitivity of ChEs to organophosphates in reptiles is similar to that reported in fish, birds and mammals (Schmidt 2003). In the case of the lizards, *Gallotia galloti* has been proposed as a sentry species for pesticides based on the activity levels of ChEs (Fossi et al. 1995; Sanchez-Hernandez and Moreno 2002), in the same way the inhibition of ChEs activity has been reported in the liver tissue of *Calotes versicolor* under the effect of pyrethroid, organophosphorate and phytopesticide (Khan 2003). This clearly indicates that the inhibition of ChEs activity in lizards can be a biomarker of these compounds, bringing support to the fact that the studied *Sceloporus* populations were not impacted by this sort of polluting agents.

The effects caused by these polluting agents on *Sceloporus* species was previously reported by (Holem et al. 2006, 2008), however the information provided was limited to the alterations of the locomotor performance, growth and general fitness. In tail samples, the AChE activity registered was greater than the activity of BChE. In contrast, the plasma AChE activity levels detected in *Gallotia galloti* were lower than the activity of BChE (Sanchez-Hernandez and Moreno 2002). Despite the scarcity of data on the physiological role of BChE, it has been pointed out that it can have a synergistic and physiological role involved in the protection of the AChE activity against neurotoxic compounds, which would contribute to explain the higher BChE activity found in the plasma when compared to other tissues (Thompson and Walker 1994).

Unlike the previous biomarkers, a noticeable difference between localities was found in the case of the CaE activity, where a higher activity was registered for the organisms of the Industrial Park. This difference was evident in spite of the interspecific variation of the CaE activity, which is generally greater than that of ChEs (Thompson and Walker 1994; Sánchez et al. 1997). A plausible explanation would be the intermittent exposure to pesticides of individuals from the Ecological Park, which could have provoked the reactivation of ChEs, once the CaE have been phosphorilized, thus generating differences which were not found in the case of ChEs (Sánchez et al. 1997; Maxwell and Brecht 2001). However, due to the lack of background information of the basal levels of these enzymes in the studied species, it is not possible to conclude that the CaE activity was inhibited in individuals from the Ecological Park. In this sense, considering that the Ecological Park is a protected pristine site where no pesticides are used to control forest pests, the biomarkers

values found in this locality could be considered as a reference and would be near the normal values. It would be expected that the variation in activity levels could be a consequence of the presence of pollutants in the Industrial Park. This contention is supported by other studies that have reported the effect of heavy metals on increasing CaE activity in spiders (Wilczek et al. 1997) and ants (Migula and Glowacka 1996). Additionally, it has been shown that the exposure to hydrocarbons increases the enzyme detoxification activity, including the CaE (Potter and Wadkins 2006; Zhang et al. 2008). This could also be the case at the Industrial Park. However, the specific factors that may have an influence on the activity of CaE in organisms of the Industrial Park cannot be determined with accuracy, since there are many isoenzymes whose physiological role in the metabolism of xenobiotics is still unknown (Wheelock et al. 2008).

Similarly to CaE, the ALP activity was different according to the location. Lower levels of ALP activity were observed in individuals from the Industrial Park, adding credence to the hypothesis that these organisms are constantly exposed to polluting agents. In fish, low levels of ALP activity has been associated to several causes such as: lead (Singh et al. 1994), toxins from vegetables (Singh and Singh 2005) and when physiological alterations are present as a result of reproductive (Trivedi et al. 2001) or renal damage (Trof et al. 2006). In contrast, the ACP activity levels did not contribute to establish differences associated to the study locations. Nevertheless, variations associated to the genders in *S. serrifer* from the Ecological Park could be the result of the reproductive condition, as reported for snakes and lizards which show ACP activity variations associated to changes in the androgen levels during the reproductive period (Deb and Sarkar 1963).

The results of GST activity was also different when considering the localities. Those organisms from the Industrial Park showed a higher activity. The GST is involved in the detoxification of xenobiotics and byproducts from lipid peroxidation and has been widely associated with exposure to metals and organic xenobiotics in insects and fish (Wilczek et al. 1997; Huang et al. 2007). Similar results have been reported in herpetofauna, in turtles (*Chrysemys picta*, *Caretta caretta*, *Chelonia mydas* and *Lepidochelys olivacea*) and in the toad *Chaunus schneideri*, the GST activity has been shown to be significantly higher in individuals in polluted sites in comparison to control sites (Attademo et al. 2007; Richardson et al. 2010). Within this context, a higher GST activity in organisms of the Industrial Park could be a consequence of industrial pollution (heavy metals and/or hydrocarbons).

The results obtained for the SOD activity also allow identifying differences in the organisms from both localities. The SOD activity is generally used as a biomarker of oxidative stress induced by xenobiotics, particularly heavy metals (Zhang et al. 2008; Costa et al. 2008). Under normal conditions the SOD, catalase and glutathione peroxidase eliminate the reactive oxygen species during the bioactivation by xenobiotics (Sk and Bhattacharya 2006). Nevertheless, the variations in the SOD activity may depend on the characteristics of each study, as inhibition, activation or even both changes have been reported in tissues from different organisms. For example, when tadpoles of *Lithobates catesbeianus* were exposed to an herbicide, the liver SOD activity increased, whereas in the muscle the activity decreased (Costa et al. 2008). Despite these considerations previous studies in fish have shown similar results. The SOD activity in liver, kidney and intestine of carps (*Cyprinus carpio*) in the Amarillo River was higher in sites affected by mixed polluting agents, including phenols, oils and ammonia (Huang et al. 2007). Also tilapias (*Oreochromis mossambicus*) exposed to cadmium displayed a significant increase in liver and kidney SOD activity (Basha and Rani 2003).

Despite the tail regeneration capability of lizards, it has been argued that the loss of the tail may have functional and physiological costs (Hare and Miller 2010). However, it has been demonstrated that the parcial loss of the tail does not have profound effects on the energetic reserves, nor in their locomotive capability (Lin and Ji 2005). In the same sense, it has also been reported that tail regeneration does not have an energetic priority over reproduction or growth, so these processes would not be affected (Dial and Fitzpatrick 1981; Althoff and Thompson 1994). As a consequence, the use of tail clips has been favored as a non-invasive alternative tissue sampling method over the conventional sampling methods, such as blood extraction or post mortem tissue sampling, particularly for small size species or for endangered species that can not be sacrificed (Ezaz et al. 2008). The uses of nondestructive sampling techniques have been developed recently in determining organic and inorganic contaminant concentrations in lizards and snakes (Campbell and Campbell 2002). Tail muscle, tail clips, skin, shed skins and blood have been used for reporting residue concentrations in lizards and snakes (Hopkins et al. 2001; Burger et al. 2005; Jones et al. 2005; Márquez-Ferrando et al. 2009). In this sense, the biochemical biomarkers can be determined with simpler and/or more economic equipment (spectrophotometer or microplates reader) that those necessary for residue analysis (e.g. atomic absorption; gas chromatography; mass spectroscopy), which can facilitate a wider use of these biomarkers as they occur in many of the aquatic ecosystems (Van der Oost et al. 2003). Overall, after analyzing the different biomarkers used in this study a clear physiological effect can be appreciated in the population of spiny lizards of the Industrial Park undoubtedly due to polluting agents such as heavy metals and/or hydrocarbons. In this

way, the present research shows the possibility of simplifying the ecotoxicology studies in lizards, by means of the use of biomarkers.

Acknowledgments The authors wish to acknowledge the program for the support of Scientific and Technological Research (Ref: PAICYT CN021-09) for financing the project.

References

Adeyemi IG, Adedeji OB (2006) Acute toxicity of acaricide in lizards (*Agama agama*) Inhabiting dog kennel in Ibadan, Nigeria: an environmental hazard in urban vector control. Environmentalist 26:281–283. doi:10.1007/s10669-006-0156-0

Althoff DM, Thompson JN (1994) The effects of tail autotomy on survivorship and body growth of *Uta stansburiana* under conditions of high mortality. Oecologia 100:250–255

Attademo AM, Peltzer PM, Lajamanovich RC, Cabagna M, Fiorenza G (2007) Plasma B-esterase and glutathione S-transferase activity in the toad *Chaunus schneideri* (Amphibia, Anura) inhabiting rice agroecosystems of Argentina. Ecotoxicology 16:533–539. doi:10.1007/s10646-007-0154-0

Baker PJ, Costanzo JP, Lee RE (2007) Oxidative stress and antioxidant capacity of a terrestrially hibernating hatchling turtle. J Comp Physiol B 177:875–883. doi:10.1007/s00360-007-0185-0

Basha PS, Rani AU (2003) Cadmium-induced antioxidant defense mechanism in freshwater teleost *Oreochromis mossambicus* (Tilapia). Ecotoxicol Environ Saf 56(2):218–221. doi:10.1016/S0147-6513(03)00028-9

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Burger J, Campbell KR, Campbell TS, Shukla T, Jeitner C, Gochfeld M (2005) Use of skin and blood as nonlethal indicators of heavy metal contamination in northern water snakes (*Nerodia sipedon*). Arch Environ Contam Toxicol 49:232–238. doi:10.1007/s00244-004-0098-9

Campbell KR, Campbell TS (2002) A logical starting point for developing priorities for lizard and snake ecotoxicology: a review of available data. Environ Toxicol Chem 21(5):894–898. doi:10.1897/1551-5028

Contreras-Lozano JA, Lazcano D, Contreras-Balderas AJ (2007) Notes on Mexican herpetofauna 10: the herpetofauna of three plant communities in the Sierra de Picachos, Nuevo León. Mexico Bull Chicago Herp Soc 42:177–182

Costa MJ, Monteiro DA, Oliveira-Neto AL, Rantin FT, Kalinin AL (2008) Oxidative stress biomarkers and heart function in bullfrog tadpoles exposed to roundup original. Ecotoxicology 17:153–163. doi:10.1007/s10646-007-0178-5

Deb C, Sarkar C (1963) Histochemistry of "renal sex segment" in garden lizard, *Calotes versicolor*. Proceedings of the National Institute of Sciences in India 29, B(2): 197–202

Dial BE, Fitzpatrick LC (1981) The energetic costs of tail autotomy to reproduction in the lizard *Coleonyx brevis* (Sauria: Gekkonidae). Oecologia 51:310–317

DuRant SE, Hopkins WA, Talent LG (2007) Impaired terrestrial and arboreal locomotor performance in the western fence lizard (*Sceloporus occidentalis*) after exposure to an AChE-inhibiting pesticide. Environ Pollut 149(1):18–24. doi:10.1016/j.envpol.2006.12.025

Ellman GL, Courtney KD, Andres V, Featherstone RM (1961) A new and rapid calorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 7:88–95

Ezaz T, O'Meally D, Quinn AE, Sarre SD, Georges A, Marshall JA (2008) A simple non-invasive protocol to establish primary cell lines from tail and toe explants for cytogenetic studies in Australian dragon lizards (Squamata: Agamidae). Cytotechnology 58:135–139. doi:10.1007/s10616-009-9182-3

Favorito R, Grimaldi MC, Coppola M, Ferrandino I (2010) Effects of acute cadmium exposure on the pituitary gland of *Podarcis sicula*. Open Zool J 3:30–36. doi:10.2174/1874336601003010030

Fossi MC, Sánchez-Hernández JC, Díaz-Díaz R, Lari L, García-Hernández JE, Gaggi C (1995) The lizard *Gallotia galloti* as a bioindicator of organophosphorus contamination in the Canary Islands. Environ Pollut 87:289–294

Hare KM, Miller KA (2010) Frequency of tail loss does not reflect innate predisposition in temperate New Zealand lizards. Naturwissenschaften 97:197–203. doi:10.1007/s00114-009-0628-4

Hare KM, Miller JH, Clark AG, Daugherty CH (2005) Total lactate dehydrogenase activity of tail muscle is not cold-adapted in nocturnal lizards from cool-temperate habitats. Comp Biochem Physiol B 142(4):438–444

Hofer R, Lackner R, Lorbeer G (2005) Accumulation of toxicants in tadpoles of the common frog (*Rana temporaria*) in high mountains. Arch Environ Contam Toxicol 49:192–199

Holem RR, Hopkins WA, Talent LG (2006) Effect of acute exposure to malathion and lead on sprint performance of the western fence lizard (*Sceloporus occidentalis*). Arch Environ Contam Toxicol 51:111–116

Holem RR, Hopkins WA, Talent LG (2008) Effects of repeated exposure to malathion on growth, food consumption, and locomotor performance of the western fence lizard (*Sceloporus occidentalis*). Environ Pollut 152:92–98

Hopkins WA, Winne CT (2006) Influence of body size on swimming performance of four species of neonatal natricine snakes acutely exposed to a cholinesterase-inhibiting pesticide. Environ Toxicol Chem 25(5):1208–1213

Hopkins WA, Roe JH, Snodgrass JW, Jackson BP, Kling E, Rowe CL, Congdon JD (2001) Nondestructive indices of trace element exposure in squamate reptiles. Environ Pollut 115:1–7

Hopkins WA, Snodgrass JW, Baionno JA, Roe JH, Staub BP, Jackson BP (2005) Functional relationships among selenium concentrations in the diet, target tissues, and nondestructive tissue samples of two species of snakes. Environ Toxicol Chem 24(2):344–351

Huang TL, Obih PO, Jaiswal R, Hartley WR, Thiyagarajah A (1997) Evaluation of liver and brain esterases in the spotted gar fish (*Lepisosteus oculatus*) as biomarkers of effect in the lower Mississippi river basin. Bull Environ Contam Toxicol 58:688–695

Huang DJ, Zhang YM, Song G, Long J, Liu JH, Ji WH (2007) Contaminants-induced oxidative damage on the carp *Cyprinus carpio* collected from the upper Yellow River, China. Environ Monit Assess 128:483–488

Jones DE, Gogal RM Jr, Naderc PB, Holladay SD (2005) Organochlorine detection in the shed skins of snakes. Ecotoxicol Environ Saf 60(3):282–287

Khan MZ (2003) Effect of agricultural chemicals on reptiles: comparison of pyrethroid and organophosphate with phytopesticide on cholinesterase activity. Pak J Biol Sci 6(9):821–825

Khan MZ, Fatima F (2002) Comparison of induced effect of pyrethroid (Cypermethrin) with organophosphate (Malathion) on GOT and GPT in liver, kidney and brain of *Calotes versicolor* Daudin (Agamidae: Reptilia). OnLine J Biol Sci 2(6):408–410

Lambert MRK (2005) Lizards used as bioindicators to monitor pesticide contamination in sub-Saharan Africa: a review. Appl Herpetol 2(2):99–107

Lin ZH, Ji X (2005) Partial tail loss has no severe effects on energy stores and locomotor performance in a lacertid lizard, *Takydromus septentrionalis*. J Comp Physiol B 175:567–573. doi:10.1007/s00360-005-0017-z

Márquez-Ferrando R, Santos X, Pleguezuelos JM, Ontiveros D (2009) Bioaccumulation of heavy metals in the lizard *Psammodromus algirus* after a tailing-dam collapse in Aznalcóllar (Southwest Spain). Arch Environ Contam Toxicol 56:276–285

Marsili L, Casini S, Mori G, Ancora S, Bianchi N, D'Agostino A, Ferraro M, Fossi MC (2009) The Italian wall lizard (*Podarcis sicula*) as a bioindicator of oil field activity. Sci Total Environ 407(11):3597–3604

Maxwell DM, Brecht KM (2001) Carboxylesterase: specificity and spontaneous reactivation of an endogenous scavenger for organophosphorus compounds. J Appl Toxicol 21(1):S103–S107. doi: 10.1002/jat.833

Mazorra MT, Rubio JA, Blasco J (2002) Acid and alkaline phosphatase activities in the clam *Scrobicularia plana*: kinetic characteristics and effects of heavy metals. Comp Biochem Physiol B 131(2):241–249

McFarland CA, Quinn MJ Jr, Boyce J, LaFiandra EM, Bazar MA, Talent LG, Johnson MS (2011) Toxic effects of oral 2-amino-4,6-dinitrotoluene in the western fence lizard (*Sceloporus occidentalis*). Environ Pollut 159(2):466–473

Migula P, Glowacka E (1996) Heavy metals as stressing factor in the red wood ants (*Formica polyctena*) from industrially polluted forests. Fresenius' J Anal Chem 354:653–659. doi:10.1007/s002161663540653

Nicholson KL, Torrence SM, Ghioca DM, Bhattacharjee J, Andrei AE, Owen J, Radke NJA, Perry G (2005) The influence of temperature and humidity on activity patterns of the lizards *Anolis carolinensis* and *Ameiva exsul* in the British Virgin Islands. Carib J Sci 41(4):870–873

Peveling R, Demba A (2003) Toxicity and pathogenicity of *Metarhizium anisopliae* var. *acridum* (Deuteromycotina, Hyphomycetes) and fipronil to the fringe-toed lizard *Acanthodactylus dumerili* (Squamata: Lacertidae). Environ Toxicol Chem 22(7):1437–1447

Potter PM, Wadkins RM (2006) Carboxylesterases: detoxifying enzymes and targets for drug therapy. Curr Med Chem 13:1045–1054

Richardson KL, Lopez-Castro M, Gardner SC, Schlenk D (2010) Polychlorinated biphenyls and biotransformation enzymes in three species of sea turtles from the Baja California Peninsula of Mexico. Arch Environ Contam Toxicol 58:183–193. doi: 10.1007/s00244-009-9360-5

Salice CJ, Suski JG, Bazar MA, Talent LG (2009) Effects of inorganic lead on western fence lizards (*Sceloporus occidentalis*). Environ Pollut 157(12):3457–3464

Sánchez JC, Fossi MC, Focardi S (1997) Serum "B" esterases as a nondestructive biomarker for monitoring the exposure of reptiles to organophosphorous insecticides. Ecotoxicol Environ Saf 37:45–52

Sanchez-Hernandez JC, Moreno B (2002) Lizard cholinesterases as biomarkers of pesticide exposure: enzymological characterization. Environ Toxicol Chem 21(1):2319–2325

Sanchez-Hernandez JC, Walker CH (2000) In vitro and in vivo cholinesterase inhibition in lacertides by phosphonate- and phosphorothioate-type organophosphates. Pestic Biochem Physiol 67:1–12

Schmidt SR (2003) Reptile cholinesterase characterization and use in monitoring anti-cholinesterases. A thesis in environmental toxicology. Graduate Faculty of Texas Tech University, Lubbock

Sciarrillo R, De Falco M, Virgilio F, Laforgia V, Capaldo A, Gay F, Valiante S, Varano L (2008) Morphological and functional changes in the thyroid gland of methyl thiophanate-injected lizards, *Podarcis sicula*. Arch Environ Contam Toxicol 55:254–261

Seebacher F, Sparrow J, Thompson MB (2004) Turtle (*Chelodina longicollis*) regulate muscle metabolic enzyme activity in response to seasonal variation in body temperature. J Comp Physiol B 174:205–210

Singh D, Singh A (2005) The toxicity of four native Indian plants: effect on AChE and acid/alkaline phosphatase level in fish *Channa marulius*. Chemosphere 60(1):135–140

Singh B, Dhawan D, Nehru B, Garg ML, Mangal PC, Chand B, Trehan PN (1994) Impact of lead pollution on the status of other trace metals in blood and alterations in hepatic functions. Biol Trace Elem Res 40:21–29

Sk UH, Bhattacharya S (2006) Prevention of cadmium induced lipid peroxidation, depletion of some antioxidative enzymes and glutathione by series of novel organoselenocyanates. Environ Toxicol Pharmacol 22:298–308

Stebbins RC (2003) A field guide to western reptiles and amphibians, 3rd ed. Houghton Mifflin, Boston, p 533

Talent LG, Dumont JN, Bantle JA, Janz DM, Talent SG (2002) Evaluation of western fence lizards (*Sceloporus occidentals*) and eastern fence lizards (*Sceloporus undulatus*) as laboratory reptile models for toxicological investigations. Environ Toxicol Chem 21(5):899–905

Thompson HM (1999) Esterases as markers of exposure to organophosphates and carbamates. Ecotoxicology 8:369–384

Thompson HM, Walker CH (1994) Blood esterases as indicators of exposure to organophosphorus and carbamate insecticides. In: Fossi MC, Leonzio C (eds) Non-destructive biomarkers in vertebrates. Lewis Publisher, Chelsea, pp 37–62

Trinchella F, Riggio M, Filosa S, Volpe MG, Parisi E, Scudiero R (2006) Cadmium distribution and metallothionein expression in lizard tissues following acute and chronic cadmium intoxication. Comp Biochem Physiol C 144(3):272–278

Trivedi SP, Kumar M, Mishra A, Banerjee I, Soni A (2001) Impact of linear alkyl benzene sulphonate (LAS) on phosphatase activity in testis of the teleostean fish, *Heteropneustes fossilis* (Bloch). J Environ Biol 22(4):263–266

Trof RJ, DiMaggio F, Leemreis J, Groeneveld AB (2006) Biomarkers of acute renal injury and renal failure. Shock 26(3):245–253

Unrine JM, Jackson BP, Hopkins WA, Romanek C (2006) Isolation and partial characterization of proteins involved in maternal transfer of selenium in the western fence lizard (*Sceloporus occidentalis*). Environ Toxicol Chem 25(7):1864–1867

Van der Oost R, Beyer J, Vermeulen NPE (2003) Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environ Toxicol Pharmacol 13:57–149

Voituron Y, Servais S, Romestaing C, Douki T, Barré H (2005) Oxidative DNA damage and antioxidant defenses in the European common lizard (*Lacerta vivipara*) in supercooled and frozen states. Cryobiology 52(1):74–82

Wheelock CE, Phillips BM, Anderson BS, Miller JL, Miller MJ, Hammock BD (2008) Applications of carboxylesterase activity in environmental monitoring and toxicity identification evaluations (TIEs). Rev Environ Contam Toxicol 195:117–178. doi: 10.1007/978-0-387-77030-7_5

Wilce MCJ, Parker MW (1994) Structure and function of glutathione-s-transferases. Biochem Biophys Acta 1205:1–18

Wilczek G, Majkus Z, Migula P, Bednarska K, Swierczek E (1997) Heavy metals and detoxifying enzymes in spiders from coal and metallurgic dumps near Ostrava (Czech Republic). Proceedings of the 16th European Coll. Arachnology Siedlce, pp 317–328

Zhang X, Yang F, Zhang X, Xu Y, Liao T, Song S, Wang J (2008) Induction of hepatic enzymes and oxidative stress in Chinese rare minnow (*Gobiocypris rarus*) exposed to waterborne hexabromocyclododecane (HBCDD). Aquat Toxicol 86:4–11