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La macrofauna edáfica, indicadora de contaminación por metales pesados en suelos ganaderos de Mayabeque, Cuba

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Scientific Paper

Edaphic macrofauna, indicator of contamination by heavy metals in livestock production soils of Mayabeque, Cuba

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ABSTRACT: A study was conducted in order to evaluate the performance of the edaphic macrofauna in livestock production soils of the Mayabeque province, Cuba, with different degrees of contamination by heavy metals. In 2012 three plots were sampled dedicated to the cultivation of pasture (*Cyperus gigantus* L. and *Cynodon dactylon* L. Pers.), with a concentration gradient of Zn lower than the intervention limit and of available Pb up to 3,5 times higher than the intervention limit; and a non-contaminated plot as control. The rainy season and the 0-10 cm depth were the most favorable for sampling in pastureland ecosystems; and the variations in taxonomic richness, total organisms, relative abundance of each taxon, population density and functional trophic groups for the contaminated ecosystems, were determined through a Union Jack sampling. The taxonomic richness and total organisms significantly decreased wen increasing the concentration of bioavailable Pb and Zn, according to the order Pastureland > Plot 2 > Plot 1 > Ceramic. All the evaluated taxa showed decreases in the relative abundance, and the density of their populations significantly decreased with the increase of the metal concentration, in the order Pastureland > Plot 1 > Plot 2 = Ceramic. The prevailing trophic groups were the predators, and the presence of decomposers diminished. The relative sampling of populations of the Diplopoda class, phytophagous insects of the Coleoptera and Hymenoptera orders and earthworms (Oligochaeta) can be sufficient to indicate the ecological status of the soils.

Keywords: *Cynodon dactylon* L. Pers., *Cyperus gigantus* L., soil organisms, lead, zinc

INTRODUCTION

Heavy metals are one of the contaminants which are more frequently found in the agroecosystems close to industrialized regions; they cause important losses in productivity, because of the toxic effect they generate on the living organisms that inhabit the zone or on the ones which are cultivated or herded in them (Socarrás, 2013).

Studies conducted in different parts of the world report that grazing animals which feed on plants (especially pastures) cultivated in contaminated sites accumulate important levels of heavy metals in the muscle, liver and milk, which can be toxic for the animal and man, last link of the trophic chain (Kodrik *et al*., 2011). Among the reported effects are: neurotoxicity, renal and hepatic failure, genotoxicity, cancer and eventually death, in the animals as well as in human beings (Fernández-Calliani, 2012; Cachada *et al*., 2013).

There is a large number of published works which evaluate the effects of toxicity for heavy metals on the diversity, performance and response of soil organisms, besides the accumulation of these elements in the animal biomass, mainly in long-life species, and the possible bioaccumulation in the trophic chain, starting from the one that shows herbivore and omnivore habits to the carnivore ones (Tack and Vandecasteele, 2003; Santorufo *et al*., 2012).

Because there is a large extension of cultivated pastures near the industrial areas and due to the complexity and the monetary value of the analytical techniques of metal detection, it is necessary to search for cost-effective alternatives which can indicate the soil and pasture status before introducing the land in question into an animal feeding scheme (Nahmani and Rossi, 2003). In this sense, zoologists have studied the performance of the edaphic macro- and mesofauna in the presence of these contaminants, and it has been proven that for certain edaphoclimatic conditions there are species and families which can indicate early the presence of contaminants in the agroecosystems; which contributes to prevent higher damage to the health and the ecosystem itself (Nahmani and Lavelle, 2002). Knowing and describing the performance of these bioindicators will allow to evaluate the risk of transference of the contaminants in a certain region and to adopt timely decisions about its exploitation.

In a country like Cuba, with important limitations of natural resources and large land extensions dedicated to pasture crops, the search for economic alternatives that allow to identify contamination problems represents a considerable contribution to food security, animal welfare and the economy of the country. The evaluation of the performance of the macrofauna populations and their main indicators can be one of those alternatives.

Due to the above-mentioned facts, the objective of the conducted study was to evaluate the performance of the edaphic macrofauna in livestock production soils of the Mayabeque province, with different degrees of contamination by heavy metals.

MATERIALS AND METHODS

The experimental work was developed in the months of February (dry season) and October (late rainy season) of 2012, in four 1-ha plots of pastures, aimed at animal consumption in the Mayabeque province, Cuba.

The climate conditions of that year in the western region of Cuba were within the average historical values of temperature and rainfall, which varied from 21,7 to 27,3 ºC and 76,4 mm of rainfall in February (dry season) and from 25,9 to 31,5 ºC and 237,5 mm of rainfall in October (rainy season), according to the data reported by the meteorological station of Tapaste.

The area of study comprised three plots (table 1) which, according to previous studies (Mesa, 2009), showed metallic contamination from the Enterprise of Soft Ceramic Adalberto Vidal, of San José de las Lajas, with 57 years of uninterrupted exploitation. The control plot (La Asunción farm) was established taking into consideration the soil type of the contaminated plots which had physical-chemical relation with the original soil and the pasture type present.

The dumping area of the enterprise and plots 1 and 2 were exploited for the grazing of small animals and cattle until 2010, when their contamination degree was determined and this activity was prohibited. There are reports of sick animals and deaths in the dumping area due to the consumption of contaminated water from the enterprise (Guzmán *et al.*, 2008). On the other hand, the area of La Asunción farm is an active grazing area for the same type of livestock.

The bioavailable content of Pb and Zn in the soil was determined according to the technique proposed by Ma and Rao (1997) in the five monoliths where the macrofauna was extracted; EDTA 0,05 mol. L^{-1}

Table 1. Chosen areas for performing the samplings.

Ecosystem (treatment)	Description	Location (reference)
Ceramic	Artificial substratum of 200 cm of thickness, without chemical-physical contact with the original soil. Original soil: Lixiviated eutric Yellowish Ferralitic. 10 % slope. Predominance of the species *Cynodon dactylon* L. Pers. (67 %), *Cyperus gigantus* L. (24 %) and *Typha dominguensis* L. (9 %).	Waste dumping area of the White Ceramic Enterprise Adalberto Vidal (Guzmán *et al.*, 2008).
Plot 1	Original soil: Ferralitic Red. At a depth of 70-80 cm it is mixed with residues. 10 % slope. Predominance of the species *Cynodon dactylon* L. Pers. (52 %) and *Cyperus gigantus* L. (48 %).	100 m downstream from the dumping area of the White Ceramic Enterprise (Mesa, 2009).
Plot 2	Original soil: Ferralitic Red. At a depth of 120 cm it is mixed with residues. Slope lower than 3 %. Predominance of the species *Cynodon dactylon* L. Pers. (65 %) and *Cyperus gigantus* L. (35 %).	300 m downstream from the dumping area of the White Ceramic Enterprise (Mesa, 2009)
Control pastureland (without contamination)	La Asunción farm. Ferralitic Red soil. Without sediments or contamination source nearby. Predominance of the species *Cynodon dactylon* L. Pers. (71 %) and *Cyperus gigantus* L. (29 %).	3 km SW of the White Ceramic Enterprise.

was used as extracting agent. The quantification was made through SP9 atomic absorption spectrometry.

In each area 10 monoliths were selected and a Union Jack sampling scheme was followed, discarding a meter of edge for each side of the field. The dimensions were 25 x 25 x 30 cm for the five monoliths of the macrofauna and 50 x 50 x 30 cm for the five monoliths of the earthworms, according to the methodology suggested by Anderson and Ingram (1993). The removal of the soil in each monolith was made at the depths of 0-10 cm and 10-30 cm.

The total organisms in each monolith were collected and preserved in 75 % alcohol (organisms with chitinous exoskeleton) and 4 % formaldehyde (earthworms). The taxonomic identification, to the family level in the verifiable cases, was made through the keys cited by Brusca and Brusca (2003).

From the data the variance homogeneity was determined through the Kolmogorov-Smirnov test, for $p < 0{,}05$.

The evaluated indicators were:

- Taxonomic richness: taken as the number of taxonomic families per ecosystem.
- Total individuals: total number of individuals captured per area.

To determine the variations in the profile and depth, independent from the metallic stress, a bifactorial variance analysis was carried out and then Duncan's test, for 95 % confidence, with the statistical program Statgraphic plus 5.0.

The differences among the means of the total individuals in the contaminated ecosystems were evaluated through a simple classification variance analysis and then Duncan's test, for 95 % confidence (SPSS 9.0); in this case the Formicidae family was analyzed separately for a better visualization of the results.

- Relative abundance: $R = \frac{n}{N} * 100$

Where:

- n: number of individuals of each taxon within the taxonomic category family.
- N: total individuals of all the corresponding taxa to the taxonomic category family
- Density: Number of individuals per m^2.
- Trophic groups: for their definition the suggestions made by Brusca and Brusca (2003) were taken into consideration.

RESULTS AND DISCUSSION

The bioavailable concentrations of Pb^{2+} (table 2) in the plots that received the influence of the residues of the Ceramic Enterprise exceeded the total intervention values for soils under agricultural exploitation, according to CETESB (2005).

The Pb^2 gradient was perfectly defined in the evaluated ecosystems, because the authors observed a non-contaminated control ecosystem (Pastureland); an ecosystem whose content is considered, according to CETESB (2005), higher than the permissible intervention limit (Plot 1); and two ecosystems that exceeded it in 2,4 and 3,7 times (Plot 2 and Ceramic, respectively).

With regards to the Zn^{2+} gradient, the concentrations did not exceed the intervention limit for any of the evaluated areas, and only the prevention limit was exceeded in the direct dumping area of the enterprise.

The Pb and Zn values in this study for the Ceramic area and for plots 1 and 2 are within the range reported by Mesa (2009) and by Regalado *et al.* (2014). It should be taken into consideration that in the case of the first

Table 2. Concentrations of bioavailable Pb^{2+} and Zn^{2+} in soils of the evaluated ecosystems.

Ecosystem (treatment)	Pb (mg kg^{-1})	Zn (mg.kg^{-1})	Interpretation
Ceramic	663,30	322,46	Pb >>> intervention Zn > prevention
Plot 1	178,76	198,47	Pb > intervention Zn < prevention
Plot 2	441,34	298,48	Pb >> intervention Zn < prevention
Control pastureland	44,32	65,78	Pb and Zn << prevention limit
Prevention value (CETESB, 2005)	72	300	-
Intervention value (CETESB, 2005)	178	450	-

two pseudototal contents are also reported. This result indicates that the measures implemented by the municipal Government and by the enterprise itself since 2010 have been able to contain the increase of contamination (maintaining the zone covered with vegetation to prevent hydric erosion and not dumping solid residues), but not mitigate it; for such reason, it is necessary to implement a recovery strategy. Similar results were reported by Duarte *et al.* (2012) when studying urban sites with different degrees of metallic contamination.

Regarding the macrofauna, in the samplings 1 365 individuals were collected included in three Phyla (Arthropoda, Annelida and Mollusca), seven classes (Insecta, Chelicerata, Malacostraca, Diplopoda, Chilopoda, Oligochaeta and Gastropoda), 18 orders and 28 families (table 3).

When analyzing the variations produced in the populations of edaphic organisms the interactions they have with other abiotic factors such as climate, sampling depth, pH, humidity, texture and management should not be forgotten (Santorufo *et al.*, 2012; Luz *et al.*, 2013). That is why it is important to discriminate between the effects caused by these factors and those brought about by metallic stress.

Table 3. Taxonomic composition of the soil macrofauna in the studied ecosystems.

Phylum	Class	Order	Family	C	P1	P2	Pas
Annelida	Oligochaeta	Haplotaxida	Glossoscolecidae		x	x	x
			Megascolecidae				x
Mollusca	Gastropoda	Archaeogastropoda	Undetermined family		x		x
	Chelicerata	Araneae	Undetermined family	x		x	x
	Chilopoda	Geophilomorpha	Ballophilidae		x		x
			Geophilidae				x
		Scolopendromorpha	Scolopocryptopidae		x	x	x
			Scolopendridae		x		x
		Spirobolida	Undetermined family			x	x
	Diplopoda	Polydesmida	Undetermined family		x	x	x
		Polyxenida	Polyxenidae		x	x	x
		Blattodea	Undetermined family				x
		Coleoptera	Attelabidae				x
			Carabidae				x
			Cerambycidae			x	x
Arthropoda			Chrysomelidae			x	
			Curculionidae			x	
			Elateridae		x	x	x
			Scarabaeidae		x		
	Insecta		Staphylinidae	x			x
		Dermaptera	Undetermined family				x
		Diptera	Undetermined family				x
		Hemiptera	Undetermined family				x
		Hymenoptera	Formicidae	x	x	x	x
		Isoptera	Termitidae				x
		Lepidoptera	Undetermined family				x
		Orthoptera	Undetermined family				x
	Malacostraca	Isopoda	Armadillidae		x	x	x

C: Ceramic, P1: Plot 1, P2: Plot 2, Pas: Control pastureland.

The bifactorial analysis (table 4) showed highly significant differences in the total organisms sampled in the control ecosystem (Pastureland), for the sampling season and depth as well for the interaction between them. The highest values were found in the rainy season in the 0-10 cm depth, for which it is suggested that when using macrofauna as bioindicator this combination of factors is used in the samplings; thus there will be a better representativeness of the different taxa and more accurate conclusions will be arrived at.

Similar results were obtained by Cabrera *et al.* (2011) and Huauya and Huamaní (2014) in Ferralitic Red soils, dedicated to cacao cultivation.

Regarding the taxonomic richness, in the control ecosystem (Pastureland), organisms from 24 families were found; in Plot 2, from six families; in Plot 1, from four families; and in the direct dumping area of the enterprise (Ceramic), from only three families. This showed a progressive decrease of the taxonomic richness following the order: Pastureland > Plot 2 > Plot 1 > Ceramic. This decrease represented 75 % of the taxonomic diversity of the control area in Plot 2, 83,4 % in Plot 1 and 87,5 % in the Ceramic area.

Nahmani and Lavelle (2002) found decreases in the taxonomic richness when comparing sites with Zn concentration gradients; however, they obtained higher richness in ecosystems with concentrations between the prevention and intervention limits than in non-contaminated sites, mainly of non-social arthropods; this does not coincide with the findings in this study. It must be stated that the sampled ecosystems were multi-contaminated and that Zn is an essential metal, with a higher tolerance range in the organisms; while Pb is not and its toxicity is higher, which influences the decrease of the reproduction rate and the viability of the offspring and the biological activity (Heddle *et al.*, 2013; Yang *et al.*, 2014).

The total indicator of individuals (fig. 1) could differentiate well the contaminated ecosystems and the pattern. In the control pastureland less than 5 % of the evaluated points showed a quantity of individuals of around 55, and the mean was 87. The contaminated ecosystems did not show differences among themselves and the number of organisms was lower than 45, with a mean of 28-33 individuals.

The performance of the Formicidae family was separately represented, because its high number of individuals, higher than the other evaluated taxa, could have masked the statistical results. In this case a progressive decrease of the number of ants was observed with the increase of the concentration of heavy metals. The Formicidae family is the prevailing group in most of the pastureland ecosystems, according to the studies conducted by Luz *et al.* (2013), and along with the Carabidae family and the Lepidoptera order are the most used taxa as bioindicators of the quality of the edaphic ecosystems (Santorufo *et al.*, 2012).

This is an indicator that can be used in a practical way, because it does not require specialized knowledge of the macrofauna; a simple global counting of the number of individuals can indicate that there are stress factors on the populations and also recommend a chemical analysis of the soils (Rousseau *et al.*, 2013).

The relative abundance is an indicator that shows as percentage the dominance of a taxon with regards to the other taxa present. All the ecosystems were dominated by the Formicidae family (Hymenoptera), with values that varied between 76,39 % as minimum (Ceramic) and 90,59 % as maximum (Control pastureland), which coincides with the results of the samplings of Cuban pastureland ecosystems conducted by Cabrera *et al.* (2011).

With regards to the other family taxa, the earthworms (Oligochaeta class) represented 15,61 %

Table 4. Total of individuals collected in different seasons and depth, in the pattern ecosystem (Pastureland).

Season	Depth	Lower limit	Higher limit	Mean
Rainy	0-10 cm	65	74	70a
	10-30 cm	19	28	24b
Dry	0-10 cm	3	12	8,4c
	10-30 cm	0	9	4,8c

Different letters indicate statistically significant differences in the interaction of both factors, in a bifactorial variance analysis, with Duncan's test for $p < 0,05$.

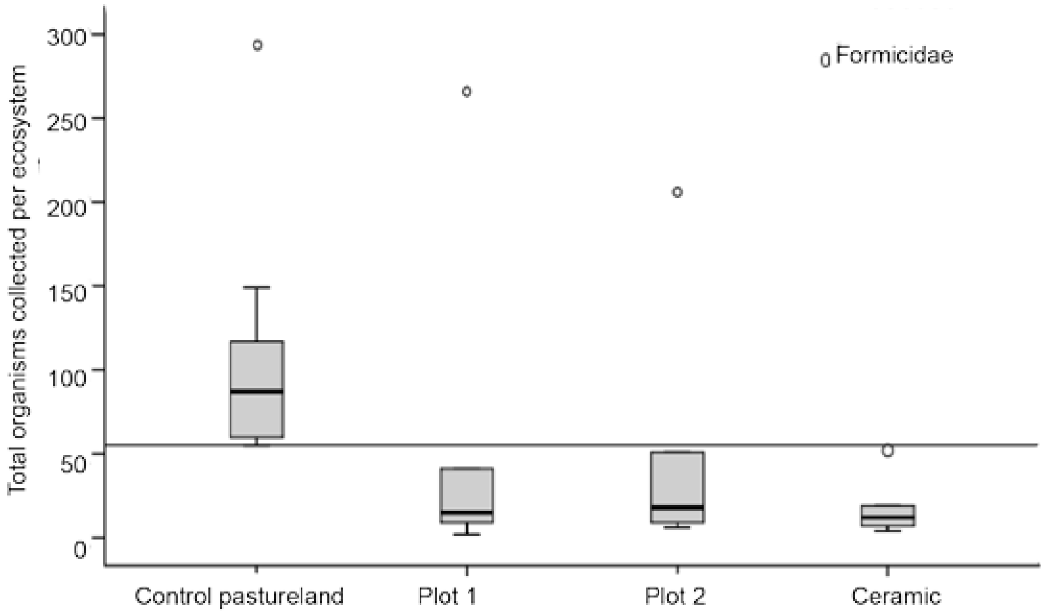

Figure 1. Total of individuals collected in the evaluated ecosystems.

Legend: the horizontal lines within the boxes show the mean of the quantity of individuals collected in 75 % of the sampling spots (without including the Formicidae family), the boxes represent the range of the number of collected individuals in 95 % of the sampled spots, and the vertical bars indicate the interval of the quantity of individuals collected in the remaining 5 % (for $p < 0,05$). The continuous horizontal line that cuts across the figure marks the minimum quantity of organisms captured in the control ecosystem.

in Plot 1 and were lower than 3 % in the others; while the families of the Coleptera order represented 15,28 % of the organisms collected in the Ceramic area and were lower than 5 % in the other ecosystems (table 5). The taxa corresponding to the families of the orders Isoptera, Dermaptera, Hemiptera, Diptera, Lepidoptera, Blattodea and Orthoptera only appeared in the control ecosystem and with abundance lower than 1 %, for which they can be classified as occasional organisms (Rousseau *et al*., 2013). A decrease of the relative abundance was observed in the taxon of the family of the Isopoda order when the concentration gradient for both metals increased.

Nahmani and Rossi (2003) referred similar results to the ones reached in this study with regards to the predominance of the families of phytophagous coleopterans in ecosystems highly contaminated by Pb, at the same time as the progressive decrease of Isopoda, Chilopoda and Gastropoda populations; and indicated that depending on the species, it is possible to find arachnids and gastropods which can serve as bioindicators. This last group was not collected in the studied contaminated systems.

The collected organisms corresponded to the four trophic groups listed in table 6, according to the criteria expressed by Brusca and Brusca (2003). Most were placed in the group of predators and represented 97,1; 96,7; 98,8 and 83,3 % of the collected individuals in each ecosystem, respectively.

It was also observed that the most sensitive organisms belonged to the trophic group of saprophytic decomposers, with a 70 % reduction in the most contaminated ecosystem (Ceramic) with regards to the control.

In the case of geophagous organisms, Plot 1 showed the highest quantity, while in the other ecosystems their populations were lower. It is considered that this result can be due to some particular ecological condition that has favored the increase of earthworm populations, such as the presence of an irrigation system in the area, which supplies an additional source of moisture, favorable factor for the development of these individuals; nevertheless, the studies conducted by Nahmani and Lavelle (2002) refer the presence of earthworm species in sites with moderate contamination.

These authors found that the prevailing coleopteran families in contaminated sites were ranked as follows: Staphilinidae-Carabidae-

Table 5. Relative abundance of the taxa studied in each ecosystem.

Taxon	Relative abundance (%)			
	Control pastureland	Plot 1 1	Plot 2	Ceramic
Diplopoda	0,23	1,95	0,50	0
Quilopoda	0,23	0,98	2,48	0
Isopoda	0,93	0,98	0,50	0
Oligochaeta	1,26	15,61	2,97	0
Gastropoda	2,94	1,46	0	0
Chelicerata	0,46	0	1,98	8,33
Isoptera	0,23	0	0	0
Dermaptera	0,30	0	0	0
Hemiptera	0,45	0	0	0
Diptera	0,15	0	0	0
Blattodea	0,23	0	0	0
Lepidoptera	0,20	0	0	0
Orthoptera	0,23	0	0	0
Coleoptera	3,24	1,95	0,99	15,28
Hymenoptera	86,72	77,07	90,59	76,39

Table 6. Total of organisms per trophic function.

Ecosystem (treatment)	Predators	Phytophagous insects	Decomposers	
			Saprophytic	Geophagous
Control pastureland	515	2	10	3
Plot 1	382	2	2	9
Plot 2	340	1	2	1
Ceramic	80	13	3	0

Curculionidae, in ecosystems which increased the bioavailable concentrations of Pb; while in this study only the Staphilinidae family was able to colonize the most contaminated ecosystem, and from it adult individuals were found (table 3). Other families of coleopterans present in Plot 2 (Pb >> intervention limit) were Cerambycidae, Chrysomelidae and Curculionidae, presumably colonizers of empty niches due to the fractures of the trophic chains (Fränzle *et al*., 2007). In addition, Feio and Dolédec (2012), Gerish *et al.* (2012) and Huauya and Huamaní (2014) stated that in contaminated soils the proportion of wide-mobility organisms increases with regards to that of the preserved ecosystems.

The trophic composition of the heavy-metals tolerant biota, according to literature, undergoes variations comparable to the ones detected in this study. Heddle *et al.* (2013) reported predominance of detritivorous groups (62 % of geophagous and 37 % of saprophytic groups) in non-contaminated soils with regards to a high incidence of zoophagous individuals (predators) in contaminated soils. Tack y Vandecasteele (2003) reported as resistant to high concentrations of heavy metals some earthworm species, certain gastropods, terrestrial hymenopterans (leaf-cutter ants and ants) and colemboles, which supports the results shown. On the other hand, Huauya and Huamaní (2014) referred that generalist species show higher environmental plasticity and are capable of colonizing degraded or contaminated sites more successfully than the organisms with higher degree of specialization.

The density of organisms maintained the following order: Pastureland > Plot 1 > Plot 2 = Ceramic (table 7), with differences of around 200 organisms m^{-2} between

Table 7. Density of organisms per ecosystem (organisms m^{-2}).

Ecosystem	Lower limit	Higher limit	Mean	Median	SD x
Control pastureland	420	460	448a	454	19,86
Plot 1	112	176	154b	164	28,56
Plot 2	16	48	31c	30	13,11
Ceramic	16	60	39c	40	19,14

Different letters indicate statistically significant differences according to Duncan's test, for $p < 0,05$.

the control ecosystem and the least contaminated one, which represented a decrease of 34,3 %; while with regards to the other ecosystems (Plot 2 and Ceramic), the density decreased between 69,1 % and 87,0 %, without differences between them, which is equivalent to a decrease of around 400 organisms m^{-2}. These results coincide with the ones reported by Feio and Dolédec (2012).

CONCLUSIONS

The total indicators of individuals and density in the rainy season and at the depth of 0-10 cm can be considered the most adequate, due to their feasibility of use and discriminating capacity. The trophic groups, the taxonomic richness and their relative abundance, although not easily measurable because they require a higher scientific knowledge, offer useful elements to determine the impact of heavy metals. The sampling (relative to a known pattern area) of populations of the families found (belonging to the Diplopoda class), of the phytophagous insects (orders Coleoptera and Hymenoptera) and of earthworms can be sufficient to indicate the ecological status of soils and their contamination degree. All this constitutes an efficacious tool to evaluate the contamination by heavy metals in Cuban pastureland ecosystems as well as for its follow-up.

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