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SOIL INVERTEBRATES- AN USEFULL TOOL IN BIOMONITORING OF HEAVY METAL POLLUTION. A REVIEW

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ABSTRACT: The atmospheric pollution and its impact on human life, increased attention paid to the invertebrates. It was determined that human intervention causes important quantitative (density) and qualitative (diversity) changes of the invertebrate populations from the affected ecosystems in comparison with those from natural areas. Studies concerning the invertebrates' usage as biomonitors had been started from 1977-1978. The lab methods became more and more modern, being used in order to determine concentrations of heavy metals from invertebrate bodies. These modern techniques are present in this paper. Analyzing the concentrations of heavy metals from invertebrate bodies from Europe and Russia, the higher values of cadmium (Cd) were identified on different species of beetles, mollusks, mites-oribatids and earthworms. High concentrations of lead (Pb) were identified on earthworms, isopods and mollusks. The mercury (Hg) was identified only on few species of isopods and millipedes. The biomonitor groups for iron (Fe) are earthworms and beetles; for zinc (Zn): earthworms, springtails, beetles, spiders, millipedes, mites, pseudoscorpions and mollusks. Millipedes and mites are efficient biomonitors for copper (Cu).

Most biomonitoring studies on invertebrates were realized on species from temperate zones, many of them being signaled also in Romania. However, the national biomonitoring studies that used invertebrates are few, in comparison with those from Europe, being necessary many researches with this topic.

Keywords: pollution, invertebrates, monitoring, heavy metal, review.

INTRODUCTION:

Increasing of the economic output and the rate of release of chemicals in nature has reached a level that is difficult to control their impact. Use and transformation of over 100 000 individual chemicals, whose current locations are difficult to establish, provided new research topics that have one thing in common: joining of fields such as ecology, physiology and chemistry (Market, 2007).

Exposure to chemicals can't be fully avoided and the determination of the low levels of pollution requires great efforts (Harpin et al., 2004; Reimann et al., 2003). Therefore, understanding, predicting and quantifying the pollution phenomena have both scientific and practical importance.

In the last decades, heavy metal contamination of the biotic component from ecosystems attracted the attention of many scientists. Using biological components as bio-indicators and biomonitors is a cheap and viable method (Hoodaji et al., 2012).

Studies have shown that not only plants and fungi are able to tolerate and accumulate heavy metals, also invertebrates. With these biological organisms can measure the quantities of heavy metals accumulated in a reasonable period of time, the economic cost being much lower cost than if it had been used chemical, analytical methods of analysis. These species are considered biomonitors. A biomonitor is the body (or part of an organism or a community of organisms) which provides quantitative information on the environment (Market, 2007).

This phenomen of the bioaccumulation has been observed on different groups of animals since 1960, when were noticed different concentrations of heavy metals in the body of mammals. The ability of terrestrial species to accumulate pollutants is different. However there is a small general classification, in which species are grouped into two categories: one that accumulates more and another that accumulates less. This classification was the first step toward developing of a new research field called ecotoxicology (Moriarty, 1983). Often chemical and analytical analyses are too expensive and complicated, biomonitors representing a viable and efficient solution that does not require sophisticated laboratory equipment.

The present review aims to provide information on the selection and use of invertebrate groups, as biomonitors of heavy metal pollution.

SOIL INVERTEBRATES AS BIOMONITORS

The edaphic invertebrate fauna includes those animals that spend their entire life in the soil or part of their development cycle. The classification criteria for the invertebrates that live in/on the ground are: body size, the capacity to adapt to the soil humidity conditions, the type of food, the connection with soil as a living place, etc. Soil invertebrate fauna has an important role in the decomposition processes of plant material, contributing to soil genesis (Coleman et al., 2004). Depending on the degree to which these invertebrates are involved in the processes of decomposition, the most important are: earthworms (Oligocheta), nematodes (Nematoda), springtails (Collembola) and mites (Acari).

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Taking into account the trophic biotic components of the debris food web, some authors estimated that over 75% of the total energy assimilated by plants is assimilated and redistributed to the ecosystem, with contribution of soil fauna. After some evaluations, the highest amount of necromass, before mineralization process, is crossing the digestive tract of soil invertebrates. Between 85-95% of the organic fractions ingested by soil fauna, are returning to this as manure. In this way, under the action of a soil fauna, a significant amount of necromass is passing through a particular phase, called coprogenous phase, the coprolites being favorable microhabitats for intense microbial processes (Mattew & Dindal, 1987; Walter & Dindal, 1987; Walter et al., 1988; Van Straalen, 1998; Walter & Proctor, 2003).

The atmospheric pollution and its impact on human life, increased attention paid to the invertebrates. It was determined that human intervention causes important quantitative (density) and qualitative (diversity) changes of the invertebrate populations from the affected ecosystems in comparison with those from natural areas (Steiner, 1995; Skubala & Zaleski, 2012; Santamaria et al., 2012; Manu et al., 2016).

The pollution damages the connections between different biotic components of the biocenosis. Invertebrates, especially those that their taxonomy, biology and ecology are well known, constitute the test-animal, which will be used more and more to highlight the functional status of soils and possible modifications due to the pollution (Walter & Proctor, 2003).

Taking account of the body size, invertebrates are classified as following:

- microfauna (species less than 0.02 mm): Protozoa and Nematoda. These live in the interstitial water from the soil. These invertebrates are used in ecotoxicology studies of the soil and have a high bioaccumulation potential. Protozoa were used for microcosm tests (Eisenbeis G., 2006).

The toxicity tests from artificial soils that have been used nematodes are used in order to provide additional information for the study of contaminants. In situ studies which use nematodes are based on the analysis of some maturity indices that bring new information on soil structure. These indices can be used for biomonitoring of conservation state or soil degradation (Eisenbeis G., 2006).

- mesofauna (species with immensions between 0.02 mm and 4 mm): Diplura, Acari, Collembola, Enchytreidae. Enchytraeids, that live in the top of soil, are the most common invertebrates, having an important ecological role in descomposing and humification. They are macrophagous species, able to process the excrement of other invertebrates (earthworms and microarthropods). Only a few ecotoxicological studies have used these species ex situ. The springtails (Collembola) and arachnids (Arachnida) are the most used groups for the ecotoxicology studies of soil (Eisenbeis G., 2006).

- macrofauna (bigger than 4 mm) : Araneida, Opiliones, Pseudoscorpiones, Chilopoda, Diplopoda, Isopoda, Lumbricidae, Pulmonata. The phytophagous and saprophagous land snails, which live on the soil, have a high bioaccumulation capacity, due to their life conditions. The oligochete annelids are consider also biomonitors. Taking into account the depth where they are found in soil, the annelids are classified as: epigeic species (in litter), endogeic species (in the top 10 cm of soil) and anecic species (in the few meters of soil). These are common species for temperate area and migrates on short distances. There are resistant and sensitive species to the pollution. Isopods are bioacumulators as well.

The pollutants affect the soil fauna directly and indirectly. The direct effects are associated with the pollutant transmission through the trophic chain, from one biotic component to another. These direct effects are: decreasing of the reproduction rate, of their life cycle and even mortality. The indirect effects are: the decreasing or disappearance of the food source for invertebrates (fungi, microfauna), changes in organic matter content and modifications of the microclimate.

Studies concerning the invertebrates' usage as biomonitors had been started from 1977-1978, observing that some arthropods (mites) are more sensible to the heavy metal pollution. These researches had been continued, till present (Williams et al. 1977; Strojan, 1978; Steiner 1995; Zaitsev and Van Straalen 2001; Skubala & Kafel, 2004; Migliorini et al. 2005). The biomonitoring studies had been realized in Europe (Netherlands, Finland, France, Poland, Belgium, Austria, Greece, Germany, England, Italy), beginning from 1984, till present, but also in Canada and Russia (Van Straalen & van Wensem, 1986; Morgan et al., 1986; Weigmann, 1995; Russell & Alberti, 1998; Cortet et al., 1999; Devkota & Schmidt, 2000; van Straalen et al., 2001; Seniczak & Seniczak, 2002; Scheifler, 2002; Skubala & Kafel, 2004; Khalil, 2009; Heikens et al., 2010; Gongalsky et al., 2010; Butovsky, 2011; Skubala & Zaleski, 2012; Owojori & Siciliano, 2012; Ardestani, 2014).

In ecotoxicology projects from Romania, the researches that used the invertebrates as biomonitors, have been started from 2007 and they have been taken into consideration only few groups as: isopods, chilopods, diplopods and thrips (Giurginca, 2008; 2010; Ion, 2008; Oromulu-Vasiliu & Bărbuceanu, 2008).

METHODS USED FOR THE DETERMINATION OF HEAVY METALS FROM SOIL INVERTEBRATE BODIES

The methods for analysis are diverse, qualitative and quantitative. The steps required for the heavy metals determination, involves three mandatory phases:

- sampling from monitored areas,
- the samples preparation for analysis - which varies depending on the requirements of laboratory instruments and the type of samples; in this stage can be found the following activities: acidification or acid digestion, filtration, preconcentration, etc.
- the analysis, which varies depending on the used equipment.

The most known equipment for the qualitative and quantitative evaluation of the heavy metals, are: atomic absorption spectrometry (AAS), X ray fluorescence (XRF); inductively coupled plasma-mass spectrometry (ICP-MS); plasma atomic emission spectroscopy (ICP-AES); ion chromatograph (IC); cold vapor atomic fluorescence spectrometry (CVAF); advanced mercury analyzer (AMA 254). Besides these, they are also used: neutron activation analysis (NAA), atomic absorption spectrometry using atomization in a graphite furnace (spectrometer 4100ZL Perkin-Elmer), spectrometer Aanalyst 300 Perkin-Elmer- flame atomic absorption spectrometry.

Over time, this methodology varied depending on the level of equipping, which became more and more modern, but also on the need to find simple solutions in comparison with the laborious methods of work in the laboratory. In addition, there are some other very important aspects concerning these modern methods: the high cost of equipment and reagents; their availability to a large volume of samples and not least their level of detection.

The lab and field methods used to determine the concentrations of heavy metals from organisms / terrestrial invertebrates' methods are varied. The collection of biological material (invertebrates) was generally performed manually: by extraction, with entomological net or by using the soil core. Sorting was done using the Tullgren method (Table 1). For the lab analysis the most used methods for the quantification of the heavy metal content was atomic absorption spectrophotometry (Table 2).

Studies regarding the usage of invertebrates as biomonitors for heavy metal pollution had demonstrated that these accumulate chemical substances through food (vegetable matter, fungi or other arthropods). More, fungivore species (mites, springtails, enchytraeids) in the polluted environment with heavy metals can change their trophical preferences, due to the modifications of the development conditions of fungi or due to the change of taste / their structure.

Table 1

Methods for collecting and sorting of invertebrate fauna

Taxa	Ecosystem	Pollution source	The collected body parts and the quantity	Sampling method	The extraction method	Lab processing	Reference
Carabidae Isopodae Lumbricidae Collembola Araneae Chilopoda	Deciduos forests (*Capinus betulus, Salix sp., Betula pendula, Alnus incana*)	Chemical industries	The whole body 1-2 individuals for large species or 10-20 individuals for those smaller.	Manual collection (for litter) and mowing with entomological net.	Tullgren method (mixture of water, glycerin and etilic alcohol).	All invertebrates were dried and kept in refrigerator. They have been weight with a Sartorius balance, till 1 μg dry weight for small invertebrates (as mites). For bigger invertebrates (worms) 1-5 mg of biological material was collected.	Van Straalen et al., 2001
Carabidae Collembola Araneae Diplura Acari Chilopoda Pseudoscorpionida	Forest with Pinus sylvestris	Mining and processing of Zn	The whole body 2 individuals for large species or 10-20 individuals for those smaller.	Manual collecting and selecting; aspiration.	Tullgren method (individuals were preserved in glass vials with wet stoppers.	All invertebrates were dried and kept in refrigerator. They have been weight, till 1 μg dry weight.	Van Straalen & Van Wensem, 1986
Acari-Oribatida	Meadow	Mining and processing of Zn	The whole body	The samples were taken with soil core, with diameter by 4,8 cm. Five ecosystems were investigated, six samples from each ecosystem.	Tullgren method	The individuals were kept in a mixture of water, glycerin and alcohol, on refrigerator.	Skubala & Zaleski, 2012

Mollusca Diptera Diplopoda Isopoda Chilopoda Lumbricidae Carabidae	Deciduous forest	Deposits with heavy metals	The whole body	Manual collecting.	The invertebrates were washed with deionized water and then introduced in immersion liquid with N_2	The invertebrates were kept in the refrigerator.	Morgan et al., 1986

Table 2:
Methods and equipment used for measurements of heavy metals concentrations from invertebrates' bodies

Equipment	Accessories	Reagent	Method	Reference
Atomic absorption spectrometry (AAS, Perkin-Elmer model 1100B), using atomization in a graphite furnace.	Graphite furnace.	Calcium lactate 12.5% (pH=7) Atropine $CaCl_2$ Acid ascorbic Molybdate	Total carbon and nitrogen concentrations were measured by burning of 1 - 5 mg duplicate samples, in a stream of pure oxygen, in combination with column chromatography and an elemental analyzer (Carlo Erba Estrumentazione, Milano, model 1106), using atropine (Merck, Darmstadt) for calibration. Determination of phosphorus was done after extraction with 12,5% calcium lactate (pH 7), measuring phosphate colorimetrically with an autoanalyzer (Skalar model SA 5100) at 880 nm, using the scorbic acid-molybdate reagent.	Van Straalen et al., 2001 Burghouts et al. 1998
Flameless atomic absorption spectrometry (PE 3030, HGA 400, AS 40)- for Cd and Pb; for Zn was used AAS (PE 4000)	Pyrex tubes L'vop platform graphite furnaces	1 M $NH_4H_2PO_4$ Ultrex- grade nitric and perchloric acid (7:1)	Each samples was dried and deposited on refrigerator, in quantities by 1 µg or 0,1 µg. The samples digestion was made using adapted method of the Bengtsson and Gunnarsson (1984), with Pyrex tubes and with ultrex- grade nitric and perchloric acid (7:1) Cd and Pb were measured using atomic absorption spectrometer (PE 3030, HGA 400, AS 40) and L'vop platform graphite furnaces. For Zn, AAS (PE 4000) atomic absorption spectrometer was used; measuring the absorption peak at 100 ml aliquots, withdraw from a titrator.	Van Straalen & Van Wensem, 1986
Flame atomic absorption spectrometry - Solar 939- for Zn Atomic absorption spectrometry with electrothermal atomization for Cd, Cu.	Analytic balance – AG-245 (Mettler Toledo)	Acid nitric Distilled and deionized water	50 individuals were analyzed, being weights three times, using AG-245 balance (Mettler Toledo), with an - accuracy of ±0.01 mg, a repetabiliy of ±0.02 mg and linearity of ± 0.03 mg. Species were dried and digested with concentrated acid nitric (Suprapur grade, Merck) and diluted with distilled and deionized water. Determination of metal concentration was by flame atomic absorption with flame (Solar 939- for zinc) or electrothermal atomization (for Cd, Cu). The wavelengths for Zn, Cd and Cu	Skubala & Zaleski, 2012

			were 213.9 nm, 228.8 nm and 324.8 nm. The atomizing temperature was 2400 °C (Cd) or 2600 °C (Cu). The injected sample volume into graphite cuvette was 15 µl.	
Atomic absorbtion spectrometry- (Pye/Unicam SP 2900; Varian-Techtron AA6)	Hydrogen lamp used to automatically compensate for nonatomic absorbtion.	„Analar" grade nitric acid	Wet digestion, using nitric acid.	Morgan et al., 1986

HEAVY METALS FROM SOIL INVERTEBRATE BODIES

The content of heavy metals from invertebrates represents the balance between the environment takeover and their disposal. If we take into consideration the studied invertebrate groups, this takeover vary a lot, being a great difference in the concentrations of heavy metals determined. Unlike organochlorine compounds, heavy metals do not accumulate along the food chain, except predatory species that feed on bioaccumulation organisms (Gongalsky et al, 2010; Van Straalen et al., 2001).

Heavy metals from the arthropod bodies were classified in two categories: those mentioned in law 104/2011 (Pb, Cd, Ni, As, Hg) and others (Table 3).

For one gram of dry weight, the higher concentrations of Cd were identified on different species of beetles, mollusks, mites-oribatids and earthworms. High concentrations of Pb were identified on earthworms, isopods and mollusks. The mercury was identified only on few species of isopods and millipedes (Table 3). The biomonitor groups for Fe are earthworms and beetles; for Zn: earthworms, springtails, beetles, spiders, millipedes, mites, pseudoscorpions and mollusks. Millipedes and mites are efficient biomonitors for Cu (Table 4).

In Romania, the study of invertebrates' biomonitors has been considered the impact of the airborne heavy metals pollutants in urban areas. Only few invertebrate groups were analysed: diplopods, isopods, chilopods and thrips (Table 3, 4).

Table 3:
The heavy metals identified in invertebrates body, according to national law no. 104/2011 (Pb, Cd, Ni, As, Hg)
(d.w.= dry weight; f.w.=fresh weight)

Taxa	Species	Pb	Cd	Hg	Country	Reference
Oligocheta - Lumbricidae	*Lumbricus castaneus* *Lumbricus rubellus* *Lumbricus terrestris* *Aporrectodea caliginosa* *Aporrectodea rosea* *Dendrobaena mammalis* *Lumbricus rutellus* *Allolobophora caliginosa*	< 0,5 µg g^{-1} d.w 8,18 µg g^{-1} d.w 12,5 µg g^{-1} d.w 1,41 µg g^{-1} d.w 126 µg g^{-1} d.w 502 µg g^{-1} d.w 696 µg g^{-1} d.w 5335 µg g^{-1} d.w	23,6 µg g^{-1} d.w 8,04 µg g^{-1} d.w 6,2 µg g^{-1} d.w 11,1 µg g^{-1} d.w 26,9 µg g^{-1} d.w 60 µg g^{-1} d.w 66 µg g^{-1} d.w 157 µg g^{-1} d.w		Russia England	Van Straalen et al., 2001 Morgan et al., 1986
Collembola	*Orchesella cincta* *Orchesella flavescens* *Tomocerus sp.* *Tomocerus flavescens* *Lepidocyrtus cyaneus* *Isotoma notabilis*	1,54 – 5,4 µg g^{-1} d.w 0,75 µg g^{-1} d.w 1,79 µg g^{-1} d.w 1,12 µg g^{-1} d.w	0,13-12,1 µg g^{-1} d.w 0,08 µg g^{-1} d.w 0,15 µg g^{-1} d.w 0,12 µg g^{-1} d.w 24,7 mg kg^{-1} d.w. 65,2 mg kg^{-1} d.w.		Russia Netherlands	Van Straalen et al., 2001 Van Straalen & van Wensem, 1986

Coleoptera	*Agonum assimile* *Agonum obscurum* *Pterostichus niger* *Pterostichus oblongopunctatus* *Calathus melanocephalus* *Notrophilus bigttatus* *Notrophilus rufipes* *Lathrobium brunnipes* *Abax sp (3 individuals)* *Agonum sp. (2 individuals)* *Calathus sp. (4 individuals)* *Carabus sp. (9 individuals)* *Leistus sp. (2 individuals)* *Loricera sp. (1 individuals)* *Nothiophilus sp. (2 individuals)* *Poecilus sp. (2 individuals)* *Pseudo-ophonus sp. (1 individuals)* *Pterostichus sp. (5 individuals)* *Perostichus madidus*	59,6 µg g^{-1} d.w 35,2 µg g^{-1} d.w 12,4 µg g^{-1} d.w 41,4 µg g^{-1} d.w 12,3 mg kg^{-1} d.w. 5,3 mg kg^{-1} d.w. 9,5 mg kg^{-1} d.w. 7,1 mg kg^{-1} d.w. 0,1 ppm d.w. - 1 ppm d.w 0,1 ppm d.w 2,9 ppm d.w - 1,7 ppm d.w 0,1 ppm d.w - - 62 µg g^{-1} d.w	9,16 µg g^{-1} d.w 2,32 µg g^{-1} d.w 5,16 µg g^{-1} d.w 7,37 µg g^{-1} d.w 10,5 mg kg^{-1} d.w. 18,2 mg kg^{-1} d.w. 21,2 mg kg^{-1} d.w. 44,3 mg kg^{-1} d.w. 3,1 ppm d.w 4,9 ppm d.w 6,7 ppm d.w 4,3 ppm d.w 7,8 ppm d.w 1,9 ppm d.w 1,7 ppm d.w 4,2 ppm d.w 3 ppm d.w 2,9 ppm d.w 5 µg g^{-1} d.w		Russia Netherlands Russia England	Van Straalen et al., 2001 Van Straalen & van Wensem, 1986 Butovsky, 2011 Morgan et al., 1986
Isopoda	*Hyloniscus riparius* *Porcellio scaber* *Philoscia muscorum* *Oniscus asellus* *Trachelipus arcuatus* *Cylisticus convexus* *Armadillidium vulgare*	2,50 µg g^{-1} d.w 22 µg g^{-1} d.w 543 µg g^{-1} d.w 813 µg g^{-1} d.w 2,04 µg g^{-1} d.w 7,46 µg g^{-1} d.w 7,5 µg g^{-1} d.w	1,55 µg g^{-1} d.w 22 µg g^{-1} d.w 57 µg g^{-1} d.w 72 µg g^{-1} d.w 0,53 µg g^{-1} d.w 0,20 µg g^{-1} d.w 0,26 µg g^{-1} d.w	0,70 µg g^{-1} d.w 0,34 µg g^{-1} d.w 0,33 µg g^{-1} d.w	Russia England Romania	Van Straalen et al., 2001 Morgan et al., 1986 Giurgincă et al., 2008
Araneae	*Pardosa sp.*	0,61 µg g^{-1} d.w	1,28 µg g^{-1} d.w		Russia	Van Straalen et al., 2001
Myriapoda	*Centromerus sylvaticus*	5 mg kg^{-1} d.w.	176,9 mg kg^{-1} d.w.		Netherlands	Van Straalen & van Wensem, 1986
	Centromerus sylvaticus	0,99 µg g^{-1} d.w	0,44 µg g^{-1} d.w		Russia	Van Straalen et al., 2001
	Lithobius forficatus	31,9 mg kg^{-1} d.w.	19,9 mg kg^{-1} d.w.		Netherlands	Van Straalen & van Wensem, 1986
	Schendyla nemorensis	2,6 mg kg^{-1} d.w.	149,4 mg kg^{-1} d.w.		Netherlands	Van Straalen & van Wensem, 1986
	Lithobius variegatus	480 µg g^{-1} d.w	52 µg g^{-1} d.w.		England	Morgan et al., 1986
	Polydesmus angustus	47 µg g^{-1} d.w			England	Morgan et al., 1986
	Megaphyllum unilineatum	9,03 µg g^{-1} d.w	0,08 µg g^{-1} d.w	0,10 µg g^{-1} d.w	Romania	Giurgincă et al., 2008
Acari-Oribatida	*Chamobates cuspidatus*	185 µg g^{-1} d.w	375 µg g^{-1} d.w		Russia	Van Straalen et al., 2001
	Chamobates cuspidatus		65,2 mg kg^{-1} d.w.		Netherlands	Van Straalen & van Wensem, 1986

	Tectocepheus velatus		2519 µg g^{-1} f.w		Poland	Skubala & Zaleski, 2012
	Punctoribates punctum		6554 µg g^{-1} f.w		Poland	Skubala & Zaleski, 2012
	Scutovertex sculptus		2089 µg g^{-1} f.w		Poland	Skubala & Zaleski, 2012
	Oribatula tibialis		1,925µg g^{-1} f.w		Poland	Skubala & Zaleski, 2012
	Peloptulus phaeonotus		5396 µg g^{-1} f.w		Poland	Skubala & Zaleski, 2012
Diplura	*Camphodea staphylinus*		141,7 mg kg^{-1} d.w.		Netherlands	Van Straalen & van Wensem, 1986
Pseudoscorpionida	*Neobisium muscorum*		155,2 mg kg^{-1} d.w.		Netherlands	Van Straalen & van Wensem, 1986
Mollusca	*Hygromia hispida*	176 µg g^{-1} d.w	25 µg g^{-1} d.w.		England	Morgan et al., 1986
	Deroceras caruanae	363 µg g^{-1} d.w.	53 µg g^{-1} d.w.		England	Morgan et al., 1986
	Deroceras reticutalum	254 µg g^{-1} d.w	37 µg g^{-1} d.w.		England	Morgan et al., 1986
Diptera	*Tipula paludosa*	439 µg g^{-1} d.w	33 µg g^{-1} d.w.		England	Morgan et al., 1986
Tysanoptera	*Frankliniella intonsa*	0,60-8,40 ppm d.w.			Romania	Oromulu-Vasiliu & Bărbuceanu, 2008
	Haplothrips niger	0,80-6,17 ppm d.w			Romania	Oromulu-Vasiliu & Bărbuceanu, 2008
	Bagnaliella yuccae	6,94-15,53 ppm d.w.			Romania	Oromulu-Vasiliu & Bărbuceanu, 2008

Table 4

The heavy metals identified in invertebrates body, other than those mentioned in the national law no. 104/2011 (Co, Cr, Cu, Fe, Mn, Zn) (d.w.= dry weight; f.w.=fresh weight).

Organism	Species	Fe	Zn	Cu	Mn	Co	Cr	Country	Author
Oligocheta - Lumbricidae	*Lumbricus castaneus* *Lumbricus rubellus* *Lumbricus terrestris* *Aporrectodea caliginosa* *Aporrectodea rosea* *Dendrobaena mammalis* *Lumbricus rutellus* *Allolobophora caliginosa*	909 µg g^{-1} d.w 825 µg g^{-1} d.w 3309 µg g^{-1} d.w 390 µg g^{-1} d.s 374 µg g^{-1} d.s	3336 µg g^{-1} d.w 385 µg g^{-1} d.w 485 µg g^{-1} d.w 480 µg g^{-1} d.w 355 µg g^{-1} d.w 621 µg g^{-1} d.w 1187 µg g^{-1} d.w 1280 µg g^{-1} d.w	19,5 µg g^{-1}d.w 11 µg g^{-1} d.w 9,3 µg g^{-1} d.w 10,2 µg g^{-1}d.w 15,5 µg g^{-1}d.w				Russia England	Van Straalen et al., 2001 Morgan et al., 1986

Collembola	*Orchesella cincta* *Orchesella flavescens* *Tomocerus sp.* *T. flavescens* *Lepidocyrtus cyaneus* *Isotoma notabilis*	699 µg g^{-1} d.w 329 µg g^{-1} d.w 560 µg g^{-1} d.w 490 µg g^{-1} d.w	793-1100 µg g^{-1} d.w 587 µg g^{-1} d.w 719 µg g^{-1} d.w 503 µg g^{-1} d.w 700 mg kg^{-1} d.w. 840 mg kg^{-1} d.w.	6,22 µg g^{-1}d.w 4,38 µg g^{-1}d.w 6,95 µg g^{-1}d.w 5,33 µg g^{-1}d.w				Russia Netherlands	Van Straalen et al., 2001 Van Straalen & Van Wensem, 1986
Coleoptera	*Agonum assimile* *Agonum obscurum* *Pterostichus niger* *P. oblongopunctatus* *Calathus melanocephalus* *Notrophilus bigttatus* *Notrophilus rufipes* *Lathrobium brunnipes* *Abax sp. (3 indivizi)* *Agonum sp. (2 indivizi)* *Calathus sp. (4 indivizi)* *Carabus sp. (9 indivizi)* *Harpalus sp. (1 individ)* *Leistus sp. (2 indivizi)* *Loricera sp. (1 individ)* *Nothiophilus sp. (2 indivizi)* *Poecilus sp. (2 indivizi)* *Pseudoophonus sp. (1 individ)* *Pterostichus sp. (5 indivizi)* *Perostichus madidus*	59,6 µg g^{-1}d.w 438 µg g^{-1}d.w. 160 µg g^{-1} d.w 210 µg g^{-1} d.w 532.2 ppmd.w 58.7 ppm d.w 333.9 ppmd.w 117.1 ppmd.w 461.2 ppmd.w 436.3 ppmd.w	140 µg g^{-1} d.w 788 µg g^{-1} d.w 126 µg g^{-1} d.w 170 µg g^{-1} d.w 1080 mg kg^{-1} d.w. 1250 mg kg^{-1} d.w. 860 mg kg^{-1} d.w. 3600 mg kg^{-1} d.w. 62.8 ppm d.w 95 ppm d.w 89.2 ppm d.w 96.1 ppm d.w 130 ppm d.w 118.6 ppm d.w 77.9 ppm d.w 118.7 ppm d.w 92.3 ppm d.w 116.2 ppm d.w 248 µg g^{-1} d.w	32,4 µg g^{-1}d.w 5,80 µg g^{-1}d.w 13,1 µg g^{-1}d.w 15,6 µg g^{-1}d.w 15.9 ppm d.w 25.8 ppm d.w 52.7 ppm d.w 16.9 ppm d.w 23.3 ppm d.w 30.1 ppm d.w 27.5 ppm d.w 16.3 ppm d.w 17.3 ppm d.w 29.5 ppm d.w	24.3 ppm d.w 29.1 ppm d.w 29.2 ppm d.w			Russia Netherlands England	Van Straalen et al., 2001 Van Straalen & van Wensem, 1986 Butovsky, 2011 Morgan et al., 1986

Isopoda	*Hyloniscus riparius* *Porcellio scaber* *Philoscia muscorum* *Oniscus asellus* *Trachelipus arcuatus* *Cylisticus convexus* *Armadillidium vulgare*	732 µg g^{-1} d.s 0,75 mg g^{-1} d.w. 1,17 mg g^{-1} d.w. 1 mg g^{-1} d.w.	25,3 µg g^{-1} d.s 1005 µg g^{-1} d.s 130 µg g^{-1} d.s 299 µg g^{-1} d.s 0,22 mg g^{-1} d.w. 0,075 mg g^{-1} d.w. 0,15 mg g^{-1} d.w.	2,96 µg g^{-1} d.s 0,18 mg g^{-1} d.w. 0,21 mg g^{-1} d.w. 0,26 mg g^{-1} d.w.	0,06 mg g^{-1} d.w. 0,083 mg g^{-1} d.w. 0,072 mg g^{-1} d.w.	0,012 mg g^{-1} d.w. 0,018 mg g^{-1} d.w. 0,017 mg g^{-1} d.w.	0,22 mg g^{-1} d.w. 0,07 mg g^{-1} d.w. 0,17 mg g^{-1} d.w.	Russia England Romania	Van Straalen et al., 2001 Morgan et al., 1986 Giurgincă et al., 2008
Araneida	*Pardosa sp.*	272 µg g^{-1} d.s	197 µg g^{-1} d.s	13,1 µg g^{-1} d.s				Russia	Van Straalen et al., 2001
	Centromerus sylvaticus		4370 mg kg^{-1} d.w.					Netherlands	Van Straalen & van Wensem, 1986
Myriapoda	*Lithobius forficatus*	582 µg g^{-1} d.w	182 µg g^{-1} d.w	5,51 µg g^{-1} d.w				Russia	Van Straalen et al., 2001
	Lithobius forficatus		2850 mg kg^{-1} d.w.					Netherlands	Van Straalen & van Wensem, 1986
	Lithobius variegatus		1608 µg g^{-1} d.w					England	Morgan et al., 1986
	Schendyla nemorensis		6050 mg kg^{-1} d.w.					Netherlands	Van Straalen & van Wensem, 1986
	Polydesmus angustus		406 µg g^{-1} d.w					England	Morgan et al., 1986
	Lithobius muticus		16,47 µg g^{-1} d.w	31,94 µg g^{-1} d.w		mg/kg		Romania	Ion, 2008
	Lithobius lucifugus		21,68 µg g^{-1} d.w	40,07 µg g^{-1} d.w				Romania	Ion, 2008
	Lithobius variegatus		470 µg g^{-1} d.w	31,7 µg g^{-1} d.w				Romania	Ion, 2008
	Megaphyllum unulineatum	0,47 mg g^{-1} d.w.	0,23 mg g^{-1} d.w.	0,96 mg g^{-1} d.w.			0,4 mg g^{-1} d.w.	Romania	Giurgincă et al., 2008
Acari-Oribatida	*Chamobates cuspidatus*	2638 µg g^{-1} d.w	545 µg g^{-1} d.w	37,4 µg g^{-1} d.w				Russia	Van Straalen et al., 2001
	Chamobates cuspidatus		5580 mg kg^{-1} d.w.					Netherlands	Van Straalen & van Wensem, 1986

	Tectocepheus velatus		2038,2 µg g^{-1} f.w	307,34 µg g^{-1} f.w				Poland	Skubala & Zaleski, 2012
	Punctoribates punctum		98,53 µg g^{-1} f.w	16,16 µg g^{-1} f.w				Poland	Skubala & Zaleski, 2012
	Scutovertex sculptus		64 µg g^{-1} f.w	1392 µg g^{-1} f.w				Poland	Skubala & Zaleski, 2012
	Oribatula tibialis		584,72 µg g^{-1} f.w	187,80 µg g^{-1} f.w				Poland	Skubala & Zaleski, 2012
	Peloptulus phaeonotus		142 µg g^{-1} f.w	73,30 µg g^{-1} f.w				Poland	Skubala & Zaleski, 2012
Diplura	*Camphodea staphylinus*		3130 mg kg^{-1} d.w.					Netherlands	Van Straalen & van Wensem, 1986
Pseudoscorpionida	*Neobisium muscorum*		4880 mg kg^{-1} d.w.					Netherlands	Van Straalen & van Wensem, 1986
Mollusca	*Hygromia hispida*		437 µg g^{-1} d.w					England	Morgan et al., 1986
	Deroceras caruanae		515 µg g^{-1} d.w					England	Morgan et al., 1986
	Deroceras reticutalum		619 µg g^{-1} d.w					England	Morgan et al., 1986
Diptera	*Tipula paludosa*		483 µg g^{-1} d.w					England	Morgan et al., 1986
Tysanoptera	*Frankliniella intonsa*		1,39-6,26 ppm d.w.	7,53-42,68 ppm d.w.				Romania	Oromulu-Vasiliu & Bărbuceanu, 2008
	Haplothrips niger		2,11-15,71 ppm d.w.	16,91-60,64 ppm d.w.				Romania	Oromulu-Vasiliu & Bărbuceanu, 2008
	Bagnaliella yuccae		1,88-16,62 ppm d.w.	34,74-44,08 ppm d.w.				Romania	Oromulu-Vasiliu & Bărbuceanu, 2008

In order to compare the level of pollution with heavy metals, using invertebrates as biomonitors, it must to take into account one principle: it will be used the same functional group, even the same species. We believe that the use of invertebrate groups as biomonitors for air pollution is a complex process, collection and species identification being steps that require time and specialized knowledge. Often the amount of dry matter of arthropod, which is necessary for the heavy metals analysis, is difficult to obtain, especially if the studies take into account the same species.

CONCLUSIONS:

Heavy metals become more spread pollutants, being a problem for both human and natural ecosystems. This is the reason why biomonitoring studies fulfill the important role of measuring contamination levels of invertebrates and thus can assess the impact of heavy metals on ecosystems. The majority of the heavy metals are found in body of invertebrates, in small concentrations. These are called microelements, having some important functions in biological processes. However, high quantities of these microelements could be found in the environment, eighter by directly pollution or through global geochemical circuits, having harmful effects on biocoenosis. Most biomonitoring studies on invertebrates were accomplished on species from temperate zones, many of them being signaled also in Romania. However, the national biomonitoring studies that used invertebrates are few, in comparison with those from Europe, being necessary many researches with this topic.

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REFERENCES:

Bengtsson G, Gunnarsson T, A micromethod for determination of metal ions in biological tissues by furnace atomic asorbtion spectrophotometry. Microchemical Journal, 29, 282-287, 1984.

Borella P, Bargellini A, Caselgrandi E, Menditt A, Patriarca M, Taylor A, Vivoli G, Selenium determination in biological matrices. Microchemical Journal, 58, 325–336, 1998.

Butovsky OR, Heavy metals in carabids (Coleoptera, Carabidae). ZooKeys, 100, 215–222, 2011.

Czarnecki S, Düring RA, Closed-vessel miniaturized microwave-assisted EDTA extraction to determine trace metals in plant materials. International Journal of Environmental Analytical Chemistry, 94 (8), 801–811, 2014.

Coleman DC, Crossley DA, Hendrix PFJr, Fundamentals of soil ecology. Elsevier Academic Press, 386 pp, 2004.

Cortet JG, Gomot-De Vauflery A, Poinsot-Balaguera N, Gomot L, Texier C, Cluzeau D, The use of invertebrate soil fauna in monitoring pollutant effects. European Journal of Soil Biology, 35 (3), 115-134, 1999.

Devkota B, Schmidt GH, Accumulation of heavy metals in food plants and grasshoppers from the Taigetos Mountains, Greece. Agriculture, Ecosystems and Environment, 78, 85–91, 2000.

Eisenbeis G, Biology of Soil Invertebrates. In: Soil Biology. Intestinal Microorganisms of Soil Invertebrates H. König, A. Varma (Eds.), Springer-Verlag Berlin Heidelberg, 484 pp, 2006.

Eens M, Pinxten R, Verheyen RF, Blust R, Bervoets L, Great and Blue Tits as Indicators of Heavy Metal Contamination in Terrestrial Ecosystems. Ecotoxicology and Environmental Safety, 44, 81-85, 1999.

Gadd GM, White C, Microbial treatment of metal pollution - a working biotechnology? Trends in Biotechnology, 11, 353-359, 1993.

Giurginca A, Murariu A, Giurginca M, Potentially Toxic Metals in the Oniscidea and Diplopoda from Bucharest. Advances in Arachnology and Developmental Biology. Papers dedicated to Prof. Dr. Božidar Ćurčić. S.E. Makarov & R.N. Dimitrijević (Eds.) 2008. Inst. Zool., Belgrade; BAS, Sofia; Fac. Life Sci., Vienna; SASA, Belgrade & UNESCO MAB Serbia. Vienna-Belgrade-Sofia, Monographs, 12, 201-207, 2008.

Giurginca A, Munteanu CM, Stanomir ML, Niculescu G, Giurginca M, Assessment of potentially toxic metals concentration in karst areas of the Mehedinţi Plateau Geopark (Romania). Carpathian Journal of Earth and Environmental Sciences, 5 (1),103 – 110, 2010.

Gongalsky KB, Filimonova ZV, Zaitsev AS, Relationship between Soil Invertebrate Abundance and Soil Heavy Metal Contents in the Environs of the Kosogorsky Metallurgical Plant, Tula Oblast. Ekologiya, 1, 70–73, 2010.

Heikens A, Peijnenburg WJGM, Hendriks AJ, Bioaccumulation of heavy metals in terrestrial invertebrates. Environmental Pollution, 113, 385-393, 2010.

Herpin U, Siewers U, Markert B, Rosolen V, Breulmann G, Bernoux M, Second German heavy-metal survey by means of mosses, and comparison of the first and second approach in Germany and other European countries. Environmental Science and Pollution Research, 11, 57–66, 2004.

Hsu MJ, Selvaraj K, Agoramoorthy G, Taiwan’s industrial heavy metal pollution threatens terrestrial biota. Environmental Pollution, 143, 327-334, 2006.

Ion M, Centipedes from urban environment. In: Species Monitoring in the central parks of Bucharest, Onete M., (Eds.), Ars Docendi, University Bucharest, 79-84, 2008.

Khalil MA, Janssens TKS, Berg M, van Straalen N, Identification of metal-responsive oribatid mites in a comparative survey of polluted soils. Pedobiologia, 32 (3), 207-221, 2009.

Mattew WR, Dindal DL, Offprints From Nutritional Ecology Of Insects, Mites And Spiders; 27. Nutritional Ecology Of Soil Arthropods. Edited By Dr. Frank Slansky Jr. and Dr. J.G. Rodriguez, pp.815-836, 1987.

Mejstrik V, Lepsova A, Applicability of fungi to the monitoring of environmental pollution by heavy metals. In: Markert, W.B. (Ed.), Plants as Biomonitors. Indicators for Heavy Metals in the Terrestrial Environment. VCH Weinheim, p. 365–378. Monitor Polski. No. 22, May 11, position 233, 1993.

Moriarty F, Ecotoxicology. The study of pollutants in ecosystem. London, Academic Press, 347 pp., 1983.

Morgan AJ, Morris B, James N, Morgan JE, Leyshon K, Heavy metals in terrestrial macroinvertebrates: Species differences within and between trophic levels.Chemistry in Ecology, 2, 319-334, 1986.

Neher DA, Campbell CL, Nematode communities and inicrobial biomass in with annual and perennial crops. Applied. Soil Ecology, 1, 17-28, 1994.

Oromulu-Vasiliu L, Bărbuceanu D, Thrips species resistant to urban pollution (Insecta; Tysanoptera). In: Species Monitoring in the central parks of Bucharest, Onete M., (Eds.), Ars Docendi, University Bucharest, 92-106, 2008.

Owojori OJ, Siciliano DJ, Accumulation and toxicity of metals (copper, zinc, cadmiumand lead) and organic compounds (geraniol and benzo[a]pyrene) in the oribatid mite Oppia nitens. Environmental Toxicology and Chemistry, 31 (7), 639–1648, 2012.

Řanda Z, Kučera J, Trace elements in higher fungi (mushrooms) determined by activation analysis. Journal of Radioanalytical and Nuclear Chemistry, 259(1), 99–107, 2004.

Reimann C, Siewers U, Tarvainen T, Bityukova L, Eriksson J, Gilucius A, Gregorauskiene V, Lukashev VK, Matinian NN, Pasieczna A, Agricultural soils in Northern Europe: A geochemical atlas. Geologisches Jahrbuch, Sonderhefte, Reihe D, Heft SD5, 279 pp, 2013.

Russell DJ, Alberti G, Effects of long-term, geogenic heavy metal contamination on soil organic matter and microarthropod communities, in particular Collembola. Applied Soil Ecology, 9, 483-488, 1998.

Santamaría JM, Moraza ML, Elustondo D, Baquero E, Jordana R, Lasheras E, Bermejo R, Arino AH, Diversity of acari and collembola along a pollution gradient in soils of a Pyrenean forest ecosystem. Environmental Engineering and Management Journal, 11, 1159-1169, 2012.

Scharenberg W, Ebeling E, Distribution of heavy metals in a woodland food web. Bulletin of Environmental Contamination and Toxicology, 56, 389-396, 1996.

Scheifler R. Gomot-de Vaufleury A, Toussaint ML, Badot PM, Transfer and effects of cadmium in an experimental food chain involving the snail *Helix aspersa* and the predatory carabid beetle *Chrysocarabus splendens*. Chemosphere, 48, 571–579, 2002.

Skubala P, Kafel A, Oribatid mite communities and metal bioaccumulation in oribatid species (Acari, Oribatida) along the heavy metal gradient in forest ecosystems. Environmental Pollution, 132, 51–60, 2004.

Skubala P, Zaleski T, Heavy metal sensitivity bioconcentration in oribatid mites (Acari, Oribatida) gradient study meadow ecosystems. Science of the Total Environment, 414, 364–372, 2012.

Steiner WA, Influence of air pollution on moss-dwelling animals 3. Terrestrial fauna, with emphasis on Oribatida and Collembola, Acarologia, 36, 149–173, 1995.

Strojan CL, The impact of zinc smelter emissions on forest litter arthropods. Oikos, 31, 41–46, 1978.

Walter DE, Hunt HW, Elliot ET, Guilds or Functional Groups? An Analysis Of Predatory Arthropods From A Shortgrass Steppe Soil. Pedobiologia, 31, 247-260, 1988.

Werner MR, Dindal DI, Nutritional Ecology Of Arthropods. Edited by the Dr. Frank Slansky, pp.815-830, 1987.

Van Straalen N.M., Evaluation of bioindicator systems derived from soil arthropod communities. Applied Soil Ecology, 9, 429-437, 1998.

Van Straalen MV, Van Wensem J, Heavy metal content of forest litter arthropodes as related of body size and trophic level. Environmental Pollution, 42, 202-221, 1986.

Van Straalen NM, Butovsky RO, Pokarzhevskii AD, Metal Concentrations in Soil and Invertebrates in the Vicinity of a Metallurgical Factory near Tula (Russia). Pedobiologia, 45, 451–466, 2001.

Walter DE, Proctor HC, Mites: Ecology, Evolution and Behaviour. Life at a Microscale. Springer – Verlag, second edition, p 447, 2003.

Weigmann G, Heavy metal burdens in forest soil fauna at polluted sites near Berlin. Acta Zoologica Fennica, 196, 369–37, 1995.

Williams ST, McNeilly T, Wellington EMH, The decomposition of vegetation growing on metal mine waste. Soil Biology and Biochemistry, 9, 271–275, 1977.

Zaitsev AS, Wolters V, Geographic determinants of oribatid mite communities structure and diversity across Europe: a longitudinal perspective, European Journal of Soil Biology, 42, S358–S361, 2006.

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