THE ROLE OF EARTHWORMS AS BIOLOGICAL INDICATORS OF SOIL CONTAMINATION

Georgescu B. C. Weber

University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Faculty of Animal Husbandry and Biotechnology. Calea Mănăştur 1-3. Cluj-Napoca. e-mail: c_e_georgescu@yahoo.com

Abstract. Earthworms are important components of the soil system. mainly because of their favorable effects on soil structure and function. In addition. earthworms help to increase soil fertility by formation of an organic matter layer in topsoil. These features. among others. have led to the popularity of earthworms as excellent biological indicators of soil pollution. Thus. earthworms are appropriate sentinel organisms to evaluate the impact of human activities to soil. The most widely studied earthworm species are *Eisenia fetida. Eisenia andrei. Lumbricus terrestris* and *Lumbricus rubellus*. Internationally standardized or academically not standardized test are described in the present article such as the nematode maturity index. the earthworm reproduction test. the lysosomal membrane stability test. immune system tests. enzymatic methods. heat-shock protein response assessment and DNA damage and alterations analyses.

Van Gestel and Van Brummelen defined a bioindicator as an organism that provides information on conditions from its environment by its presence or absence and by its behavior. Several species play the role of biological indicators: invertebrates. amphibians. fish. birds and mammalians.

On long-term. soil exploitation exerts a deleterious effect on terrestrial ecosystems. The most important factors involved in soil quality alteration are erosion. the loss of organic matter and chemical contamination.

In soil. earthworms are common components of the invertebrates population and are widely used as bioindicators of soil quality. Earthworms are important components of the soil system. mainly because of their favorable effects on soil structure and function. Their burrowing and feeding activities contribute notably to increased water infiltration. soil aeration. and the stabilization of soil aggregates. In addition. earthworms help to increase soil fertility by formation of an organic matter layer in topsoil (Georgescu et al. 2002). These features. among others. have led to the popularity of earthworms as excellent biological indicators of soil pollution. These organisms ingest large amounts of soil. or specific fractions of soil (i.e.. organic matter). thereby being continuously exposed to contaminants through their alimentary surfaces. Moreover. several studies have shown that earthworm skin is a significant route of contaminant uptake as well (Sanchez-Hernandez et al.. 2006).

Earthworm species such as *Eisenia fetida* are representative for the soil fauna and earthworms. representing the standard test organism used in terrestrial ecotoxicology in the European Community. Other species used for the study of soil contamination. especially under experimental conditions are *E. andrei* and *Lumbricus rubellus* (Spurgeon et al.. 2000). It was observed that *E. fetida* is less sensitive to pesticides.

Tests on earthworms aim to evaluate their response at the individual or population level (community census analysis. nematode maturity index). to assess the effects on worms reproduction. to determine cellular and subcellular functions (lisosomal stability). to evaluate the activities of enzymatic systems or the function of the immune system. to establish heat-

shock protein induction and to analyse DNA alterations or the expression of specific genes (annetocin) (Ricketts et al.. 2004).

Some of these tests (e.g. reproduction tests) are standardized at the international level. being recognized and promoted by international organizations (OECD – Organization of Economical Cooperation and Development. ISO – International Organization of Standardization. BSI – Britanic Institute of Standards) aiming to elaborate international guidelines on environment quality assessment. Others. although not standardized internationally are developed and applied in several research programmes on soil contamination.

Soil invertebrate communities are characterized by a diversity of species. *Community census analysis* aims to establish changes in a soil ecosystem by the analysis of the composition of the communities of a particular organism group in order to assess the effects of chemical contaminants. The researches have focused on changes in the dominance structure and diversity responses of specific groups such as earthworms. ants. spiders. molluscs or springtails.

The *nematode maturity index* (nematode taxa = earthworms. isopodes. spring tails. carabids. orbatids and spiders) is a measure of the impact of soil contamination on the nematode fauna. Nematodes are categorised into five groups representing different life-history and ecological strategies. The categorisation is based on the position of the genera within the continuum between species typical of variable environments (t-selected) and species typical of stable environments (k-selected). The colonisers (t-selected) with short life cycles. high reproducton rates and high colonisation ability are weighted as one. and persisters (k-selected) with long life cycles. few offspring and low colonisation ability are weighted as five. The MI is calculated as the weighted mean of the consituent nematode taxa. Changes in the MI have been observed after pesticide use (Ruess et al.. 2001). nitrogen addition and metal contamination (Ni. Cu. Zn).

The earthworm reproduction test with Eisenia fetida/Eisenia andrei aims to assess the impact of soil contaminants on sublethal parameters in earthworms. Endpoints include reproductive parameters (cocoon production per adult per week. juveniles hatching per adult per week and cocoon viability) and weight change and feeding activity of adults. During the test. adult. mature worms are exposed to different concentrations of a substance (pollutant) in a standard test soil; when field soils are used. homogenised and air-dried soil samples are sieved. added to the test chamber and brought to a given moisture content. Ten acclimatised individuals are added to each vessel containing 500g dry weight of the selected soil. Growth effects and mortality are determined after four weeks and effects on reproduction are assessed after other four weeks of exposure. The assay has been used to measure the effects of a wide range of chemicals such as metals (Lock et al.. 2001). pesticides (Kula et al.. 1997) or energetic compounds. Recent reports on earthworm ecotoxicology demonstrated that the test can be used to detect the effects of organic compounds. herbicides and polycyclic aromatic hydrocarbons. Earthworm tests can be influenced by soil factors. For example. low pH is known to reduce reproduction in worms as compared to neutral soils. In addition. use of a suitable control soil is essential.

Lysosomal membrane stability assessment is a test on lysosomal membrane fragility caused by pollutant exposure. At the subcellular level. the lysosomal system has been identified as a target for toxic contaminants. The test involves the binding of neutral red in the lysosomal matrix as a measure of cell viability. Therefore. cells are isolated from body fluids or body tissues and placed on microscope slides suspended in physiological Ringer solution.

A dilute solution of neutral red dye is added to the suspension and covered with a cover slip. The slide is then scanned using a light microscope and the number of cells with leaked lysosomes (stained red) and the number of cells remaining unstained are counted.

When 50% of the cells are stained. the time since the dye was added is noted and this is the neutral red retention time (NRRT). Lysosomos are sensitive to different organochlorines. In earthworms. NRRT has been used to assess Cu contamination (Maboeta et al.. 2004). with a dose-response relationship between metal concentrations and the NRRT in *L. rubellus* and *E. andrei*. Similar data were reported in earthworms for Ni. Zn. Pb. Cd. mixtures of metals. pesticides and TNT.

Immune system activity tests evaluate the impact of soil contamination on different types of immunological function in earthworms. Immune system studies in earthworms have focused on the determination of following parameters: phagocytosis. the ability to reject allo- and xenografts and to perform wound healing. production of reactive oxygen species. natural killing cytotoxicity. elimination of non-pathogenic bacteria and antibodies production. The most used compounds in tests with earthworms are PCBs (Cikutovic et al.. 1999). metals (Cu. Cd. Hg. Pb. Zn). organic compounds (pentachlorophenol) and several pesticides (Bunn et al.. 1996). Until know. it appears the *L. terrestris* is the only field-dwelling species in which these immune parameters have been measured.

Measurement of the activities and quantities of enzymes involved in the mixed oxidase system (MFO) is a very sensitive test given the high sensitivity of these enzymes to several contaminants. On the other hand. MFO investigation is characterized by a low specificity. The response of the MFO system to a organic pollutant is examined by determination of its components such as: - the cytochrome P-450. the activity of ethoxyresorufin-O-deethylase (EROD). of ethoxycoumarin-o-dealkylase (ECOD). of aryl hydrocarbon hydroxylase (AHH) and NADPH cytochrome c reductase.

Commonly. the activity of these enzymes is measured by fluorescence. absorbtion. luminiscence. immunologic or enzymatic assays. Modern techniques are based on molecular biology methods such as mRNA quantification or enzyme protein isolation.

The most detailed investigations of P-450 activity in soil invertebrates has been in earthworms. The P-450-dependent monooxygenase system was isolated and characterized in *L. terrestris* and *E. fetida*. ECOD activity was described *in L. terrestris*. *L. rubella* and *E. andrei*. however. no EROD activity was observed in earthworms. These discrodances are partly explained by experiments performed on earthworm fractions and different homogenates. P-450 levels are influence by biotic factors (gender. reproduction. developmental and nutrition status) and abiotic factors (temperature and photoperiode). After exposure to heavy metals. a inhibition of P-450 activity has been documented. This may be important when mixtures of pollutants are present.

In other assays the activity of *glutathione-S-transpherase* (GST). another enzymatic detoxification system. is well documented in terrestrial nevertebrates. In earthworms increased levels of GST were seen after organochlorines such as aldrin. endosulfan and lindan (Hans et al.. 1993). Unfortunately. to date. 5-6 substrate-specific GST izoenzymes habe been described in *Eisenia* species.

Measurement of the activity of *antioxidant enzymes* such as superoxiddismutase (SOD). catalase (CAT). peroxidase (Px) and glutathione reductase (GR) evaluates the influence of contaminants on the antioxidant capacity of earthworms. Antioxidant activity has been determined in *E. fetida* after exposure to Pb and Ur. both enzymes inhibiting CAT activity without influence on SOD (Labrot et al.. 1996). SOD and CAT activities were not induced in earthworms *E. veneta* and *E. andrei*. after exposure to heavy metals and herbicides (paraquat).

Inhibition of acetylcholinesterase (Ach) and cholinesterase (ChE) activity is a well known effect of organophosphate pesticides and the degree of inhibition is directly linked to the neurotoxicologic action of these pesticides. Inhibition of Ach and ChE activity by organophosphate pesticides and carbamates has been well documented in earthworms (Booth

et al.. 2000). with sublethal effects. Although it is often thought that Ach measurements are specific for some pesticides. research has been shown that heavy metals (Pb) may inhibit Che activity in *E. andrei in vivo* and *in vitro* (Labrot et al.. 1996); on the contrary. others found no inhibitory effect on ChE activity in *E. andrei* (Saint-Denis et al.. 2001).

The study of heat-shock proteins (HSP) responses aims to evaluate stress protein induction as a result of denaturation of proteins caused by exposure to contaminants (Georgescu et al.. 2004). Based on their molecular weight. stress proteins are divided in several families: low molecular weight HSP (15-40 kDa). mitochondrial or cytoplasmic HSP-60 (MW 58-60 kDa). HSP-70 (MW 66-78 kDa). HSP-90 (MW 83-90) and high molecular weight proteins (MW 100-110 kDa).

Although highly sensitive. HSP determination has low specificity. In earthworms. HSP-70 induction by thermal stress has been demonstrated for *E. fetida*. In *L. terrrestris*. heavy metals and pentachlorophenol contamination induced HSP-70 (Nadeau et al.. 2001). In *L. rubellus*. HSP-60. -70 and -90 induction in a dose-response manner has been documented in metalliferous soils (Marino et al.. 1999). Moreover. transfer from a clean soil to a metalliferous soil rapidly resulted in overexpression of HSP in earthworms (Marino et al.. 1999).

Exposure to genotoxic chemicals from soil may induce DNA damage and alterations in earthworms. Either they bind covalent to DNA forming „DNA adducts" or cause strand brakage or they decrease the level of methylation of a specific base.

DNA alterations were found in earthwoms such as *L. terrestris* (DNA adducts and strand breakage). *L. castaneus* (strand breakage) and *E. fetida* (strand breakage) (Saint-Denis et al.. 2000. Zang et al.. 2000) exposed to dioxin and its derivates. soil contaminated with organic pollutants (coke. aniline. benzen etc.) and X-rays.

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