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AN INTEGRATED BIOINDICATION SYSTEM APPLIED TO SOIL POLLUTION ASSESSMENTS: FROM EARTHWORMS TO ECOSYSTEMS

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Abstract

The proposed bioindicator system is based on an integration of the information gathered on earthworms in polluted sites. This integration deals with pollutant effects on survival (presence-absence), tissue concentrations (body burden of heavy metals, PCBs, PAHs, genotoxics, *etc.*) and on effects on the earthworm community. The integration deals also with pollutant assessment on the ecosystem resulting from community changes. These ecosystem assessments concern physical (erosion, overflow, drainage, soil stability), chemical (carbon, nitrogen cycles, pollutant fate, *etc.*) and biological properties (food chains, biocontaminations, *etc.*). The bioindicator system integrates these elements by modelling and a description of the state-of-the-art in an Explained Knowledge Dispenser. With this approach, up-to-date knowledge could be accessible and shared by integrology between the various research teams and decision-makers. It will allow to make predictions *versus* time, in a falsifiable manner allowing permanent improvements.

1. Introduction

Framework. A pollutant is a substance, simple or complex, having ecotoxicological effects, *i.e.* a toxicity on organisms living in ecosystems. Earthworms constitute the main animal biomass in our terrestrial ecosystems (110 g live weight m^{-2} in France; n = 62). They are present nearly everywhere and, depending on their ecological categories, dwell in almost all soil compartments with living components, from the epiphytic soils in tropical rain forests to the deep soil layers (2 m or more). Therefore earthworms are good candidates for assessments.

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To assess a pollutant, we must observe in soils its effects on ecosystems and human welfare. This is not enough. To assess pollutant risks, *i.e.* biological and ecosystemic disorders, the information gathered must be ordered and accessible to other specialists and decision-makers. To order knowledge two linked backgrounds are needed: modelling and integrology.

Modelling. This has been improved since the IBP [1], but drawbacks have led me to formalize model parameters: 1) The model components must be measurable in true systems, *i.e.* (agro)ecosystems, 2) Results must be falsifiable in ecosystems, 3) The model must be transposable *(i.e.* usable in a lot of ecosystems with a minimum of local variables), and 4) The model must be a synthetic tool, *i.e.* designed to be, simultaneously, a submodel of ecosystems and to integrate submodels fitted to some of its subcomponents.

Such a model was designed in 1975 and named REAL (Rôle Écologique et Agronomique des Lombriciens = Agronomical and Ecological Earthworm Role) [2, 3, 4], see Fig. 1. This conceptual model currently has numerous submodels (numerical, qualitative and fuzzy models). The effects of pollutants are registered at the individual level: stage, state, species, and if needed, body and *endentere* chemical composition (*endentere* = digestive tract content). In a given site these data are aggregated to describe effects on population and ecological categories. The ecological category concept [5, 6] allows to transpose REAL from one site to other sites, with changes in soil, species, climatic and human management conditions. Only local population studies and, if possible, soil temperature and moisture are needed to feed the model. REAL allows for the description of changes in ecosystem function in terms of physical variables (water infiltration rate, rate of soil stable crumb formation) [7, 8, 9], chemical variables, especially nitrogen dynamics [10, 11, 12] and heavy metal content of earthworms as food for other animals [13, 14, 15]. Presently, REAL does not allow for the prediction of rates of population restoration and food chain transfer of contaminants, because of the lack of demographic modelling.

Integrology. The second background, integrology, [16] was founded when the concept of **relation,** which has been intensively used in Relational Data Bases since 1984, met clear ecological concepts as DICs, prelevat, referender, described elsewhere [4] (Ecological means here globally biophysicochemical concepts, not biological or chemical or physical ones). This was intensively improved and used for an integrated management of biophysicochemical data (with no discipline borders) especially in the Relational Data Base BASECOL of ECORDRE, where billions of data (DICs) belonging to more than one thousand variables are integrated and automatically accessible [4, 17].

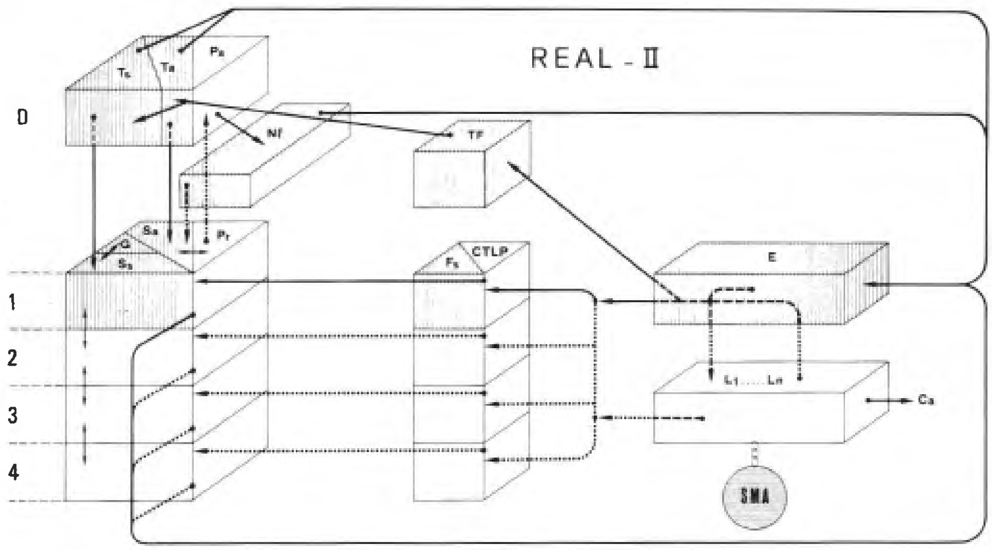

Figure 1. The REAL Model describes the roles of earthworms in ecosystems, which depend of the population levels (L_1, L_2, ..., L_n), affected or not by pollutants. Earthworms act on ecosystems by ingestion of: 1) mineral (Ss) or organic (Sa) soil, creating burrows (G), 2) their old faeces (Ts and Ta), and 3) the litter (Nl). The digestion of the endentere (E = digestive tract content) produces assimilation to L or faeces production (Fs) with a quick transfer to plant (Pr, Pa), through a transitory compartment (CTLP). This function varies with soil layers (1, 2, 3, 4, *etc.*) and the level of earthworm activity described by the submodel of activity (SMA). The pollutant body burden is transfered to predators (Ca). From [4].

More recently, the concept of relation became practicable to interpret DICs at all levels of cognition (from very tiny and sophisticated focused studies to interpolations, from highly validated results to risky falsifiable hypotheses). The description of knowledge elements integration is in progress in an Explained Knowledge Dispenser, ECOGNIT of ECORDRE, which includes a subdivision called ROLUMBRIC. We are describing, in ROLUMBRIC, REAL and its relation with assessments of pollutants. The EKD ROLUMBRIC [18] will give in full extended access to interpreted knowledge, then to the process of interpretation. It opens to an on-line knowledge service with users (*i.e.* other specialists or decision makers). Users will be able to use their local data, to ask for explanations, and make criticisms on ROLUMBRIC. ROLUMBRIC, of ECORDRE, is a tool which will be accessible on INTERNET soon.

2. The proposed new bioindicator system

2.1. PRINCIPLES

The proposed new bioindicator system is based on two principles:

1) *It is an open svstem of information*, convivial, interactive and giving access to knowledge in a way adapted to users. Giving elements of knowledge, these elements are selected by each user for its needs and are not served pre-structured, following another need or mood.

2) *It is not pre-oriented* by a favored hypothesis: the bioindicator is not to be used to indicate definitively something (example: the presence of this species means the soil is clean), but it is just used for the information it bears including temporary proposed interpretations.

Interpretations are temporary, or extemporaneous, because they are always submitted to criticism and reinterpreted for improvements. A bioindicator indicates one or several properties as hypotheses not as absolute truths (there is no room for truth in science); these hypotheses, which depend widely on local conditions, must be used carefully, *i.e.* knowing elements of knowledge used to build them and assessing the level of uncertainty they bear. For this reason a bioindicator here is in the first place a bio-informant.

The bioinformation gathered on earthworms is managed following integrological principles, including modelling, as indicated as fundamental backgrounds above.

2.2. INFORMATION DERIVED FROM EARTHWORM ECOLOGY

The information derived from earthworm ecology is of three types.

1) The direct information from them in ecosystems (= their state variables) allows **biomonitoring**, including in experimental plots.

2) The integrated information on their relations with other (agro)ecosystem components allows **environmental assessments**.

3) The modelling versus time of population changes (*i.e.* demographic and health state changes) and their related ecosystem consequences, allow **predictions**.

2.3. BIOMONITORING

Collection of knowledge. The quality of the information gathered from the field, using appropriate sampling methods and techniques depends on the objective of the study. The precise methodology has been described elsewhere [4, 19, 20].

The sampling of earthworms could be organized to get **information on individuals** and on other appropriate variables (soil, plants, *etc.*). This is made efficiently by punctual methods (linking of the various prelevats giving informations by a common unique spatio-temporal point, see [19]) and by formless prelevats (a prelevat is a formalized sample unit, see [4, 19]). This implies, for example, sampling by means of a spade in a sample unit designed in agreement with the features of the environment and not fixed by the standardized dimensions of the prelevat.

The sampling of earthworms could be designed to get, in addition, information on **community level and structure**. To obtain this information, it is necessary to use: 1) the stational method with euclidian (or cartesian) prelevats, *i.e.* unit samples having tridimensional (or bidimensional) size dimensions. These dimensions allow further interpolations at station (field) level, and 2) a quantitative extraction technique of earthworms from soils. The different techniques, either physical, ethological or physical, are tedious and time consuming, and their efficiency depends on local factors [20, 21]. This stational method associated with quantitative extractions works well with plot studies.

The punctual method associated with formless prelevats is far more flexible, efficient and cheap than the stational method with euclidian prelevats and should be used if knowledge about community structure and level is not absolutely needed. "Intermediate" practices *(e.g.* punctual method with euclidian sampling) must be discarded: they increase cost with no gain of information.

Analysis of prelevats must be made on each individual for general features (stade, state, species, weight, shape, *etc.*). Body chemical and molecular biochemistry analyses suggest it is important to clear the digestive tract. Various techniques (dissection, cellulose or levilite feedings are discussed elsewhere: [22, 23]).

All analytical data must be registered and managed, if possible, in an accessible Relational Data Base linking each DIC (date, initial and controlled) to other DICs to connect them to the five referenders: time, space, composition *(e.g.* characteristics), protocol (of analyses and observations) and observer (who did it, for who, data gathering).

The interpretation of bioindications. The presence of a susceptible taxon can indicate a clean soil if the taxon observed is well known to be susceptible to pollutants [24]. The reverse (absence) is just an assumption of risk: the absence could be because of another factor, such as an inadequate soil pH.

Earthworms eat soil components: minerals, dead organic matter from plants, animals and microorganisms. They digest and assimilate from this "sample". This sample is taken from the "live" part of the soil *i.e.* the top soil layers, the rhizosphere, and the burrows deeper in the soil. The sample does not deal with the majority of deep horizons which are inert (with no life activity). Earthworm have quick turnover of nutrients (10 % per day of their nitrogen is assimilated and excreted), and their body levels of pollutants reflect, most probably, the true ecotoxicological level of the soil (= the true bioavailable fraction of pollutants). A toxicological level is related to life and not to an unassimilable fraction as for soil analysis [15]. Earthworms are biosampling for us the biocirculating fraction of pollutants. This can be determinated for heavy metals [25, 26, 27], PCBs [23, 28, 29], PAHs [28, 30] and, more recently, for genotoxics by subquantification of adducts on earthworm DNA [31]. The level of these biorisks could be mapped or followed *versus* time.

The quantitative impact of a pollutant could be measured by the estimation of community levels and structures subjected to soil contamination either intentionally *(e.g.* field plot) or accidentally, by comparison with a non-contaminated soil. That is the unique ecotoxicological trial we have. Changes in population levels allow for estimation of most ecological consequences.

2.4. ASSESSMENTS OF CONSEQUENCES

The quantitative impact of a pollutant could be quantitatively measured by field or plot comparisons of earthworm population size. These population changes could be related to changes in ecological categories, and the latter changes reported to the earthworm functions estimated by REAL submodels. These changes could be transitory: 1) if the populations are not totally destroyed and an inoculum survives to restore the population, or 2) if the pollutant is ephemeral (degradable, *etc.*). The assessment of soil restoration depends on our ability to predict both pollutant persistence and the capability for a particular population to recover (see section 2.5.: Predictions).

Changes in earthworm communities may be evaluated for three types of consequences.

Soil physics. Especially soil stability and vertical water infiltration are important in this respect. In France, a mean population of 100 g m^{-2} live earthworms produces 30,000 g m^{-2} dry weight of stable crumbs and preserves a mean infiltration rate of 160 mm of water per hour, *i.e.* about one fifth of the annual rainfall [7, 8, 9].

Ecosystem chemistry. Especially the nitrogen cycle is of importance here. Direct soil observations have allowed us to estimate, for a mean population in France, an ingestion rate of 23,000 g N m^{-2} per year, with an excretion rate of 5300 g N m^{-2} which is almost totally assimilated by plants within a few days during the growing season while only 30 % of it is assimilated in September [10, 11, 12]. Effects on the carbon cycle are not so well described [10], but processes have a rate which is 4 to 5 times higher.

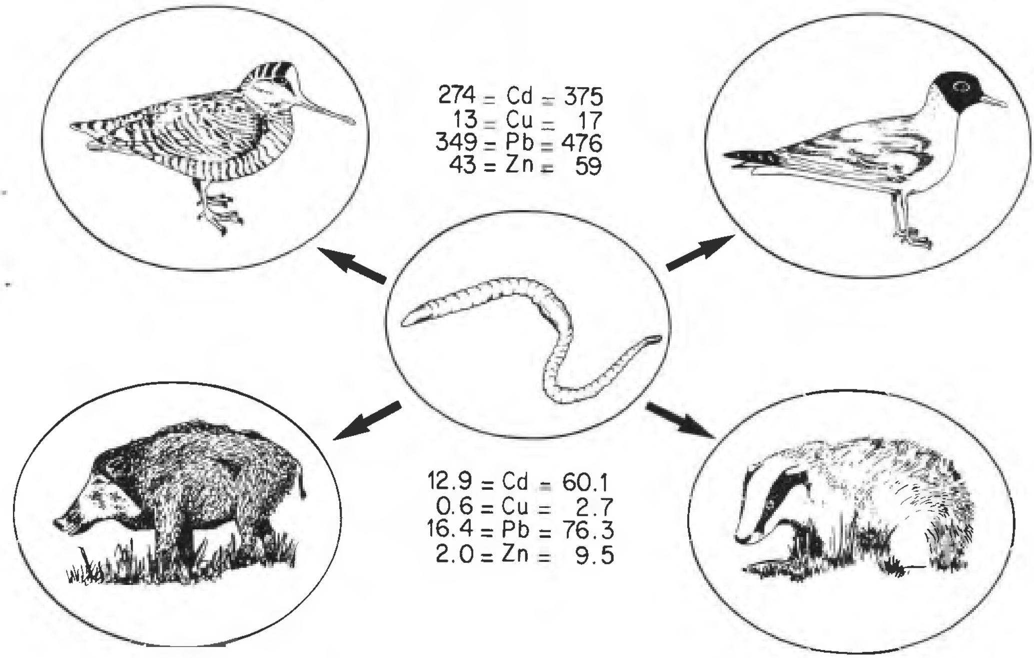

Figure 2. Transfer of heavy metals from earthworms to four important predators: the woodcock, the black-headed gull, the pig and the badger. For each predator, the number indicates the ratio between the estimated daily consumption of metals and the maximum acceptable daily intake for the human population. The data are estimated from 126 samples taken in South-East France [37].

Biological consequences. These are are more poorly documented. A lot of microcosm or pot studies demonstrate the great effect of earthworms on the other two major biological components of ecosystems: plants and microorganisms [32, 33]. Direct evidence was introduced early by the description of fertile ecosystems with mull humus type and earthworms as opposed to poor soil with mor humus type and no earthworms [34] or only epigeic species [35]. This indirect evidence could be hardly quantitatively linked to earthworm level changes.

Earthworms play an important role in food chains of numerous vertebrates by serving as their prey [36]; the consequences of heavy metal body burdens in the diet of four species (two birds and two mammals) have been estimated [38], see Fig. 2. These data show that earthworm predators consume metals at rates exceeding the acceptable daily intake for humans by a factor of 2 to 476, depending on the metal and the predator.

2.5. PREDICTIONS

To predict it is necessary to have a model describing real systems (= ecosystems) *versus* time. The proposed bioindicator system is linked with the conceptual model REAL (Fig. 1). As illustrated in Fig. 3, it can be transposed from field to field and to different earthworm community states, depending on pollutant effects, due to a general method of extrapolation using: 1) local population variables, 2) local soil moisture and temperature data, and 3) the latitude situation to calculate day-length [4]. The various quantitative (mathematical) or qualitative submodels of REAL are linked and described in the EKD ROLUMBRIC (EKD = Explained Knowledge Dispenser, an on-line knowledge service). Presently, two major drawbacks remain: 1) the absence of integration of our knowledge on the estimation of the pollutant persistence, and 2) the absence of demographic models predicting the earthworms' ability to restore their population levels after pollution exposure. Despite the use of various matrix models (Leslie, Lefkovitch, Lexis, Hadjibiros) [38] and the management of the greatest earthworm population study *versus* time [20] we were unable to produce a demographic model falsifiable based on field data. The improvement of field ecophysiological models [39] and fuzzy modelling (see [4], and G.P. Stamou, chapter 6 of this book) will lead to such falsifiable models. At present, we must sample *versus* time to follow the community restoration (T in Fig. 3).

Earthworms are not only used as bioindicators in ecosystems but also as tools in ecotoxicity tests, to provide decision makers with scientific information supporting the homologation of new chemicals before marketing [40], or to manage pollutants, such as industrial waste [41] prior to disposal or land-spreading. The bioindication system could be completed by developing links with these laboratory tests.

3. Example of application

The use of earthworms as bioindicators for the three aims described above (biomonitoring, impact assessment, prediction) is well accepted and these animals are commonly used for the assessment of soil pollutants. Examples of applications, at the research level of implementation, are numerous and are well documented in classical textbooks [32, 33, 42, 43].

Despite the relatively high number of studies this tool is poorly used in pollution assessment because these studies are scattered in the literature and are poorly accessible due to a lack of integrology [16], blocking the sharing and improvement of the new bioindicator system proposed here. In fact, this new system forces us to integrate knowledge *versus* conceptual models, such as REAL, which must be criticised and improved in real time.

The integration, *i.e.* their quick access, of the elements of knowledge we are gathering around the world about the theme of "earthworms as pollutant bioindicators" must be first improved. The gathering of elements of knowledge without their full access is similar to the collection of water with a sieve: while we are collecting the elements we lose the resource.

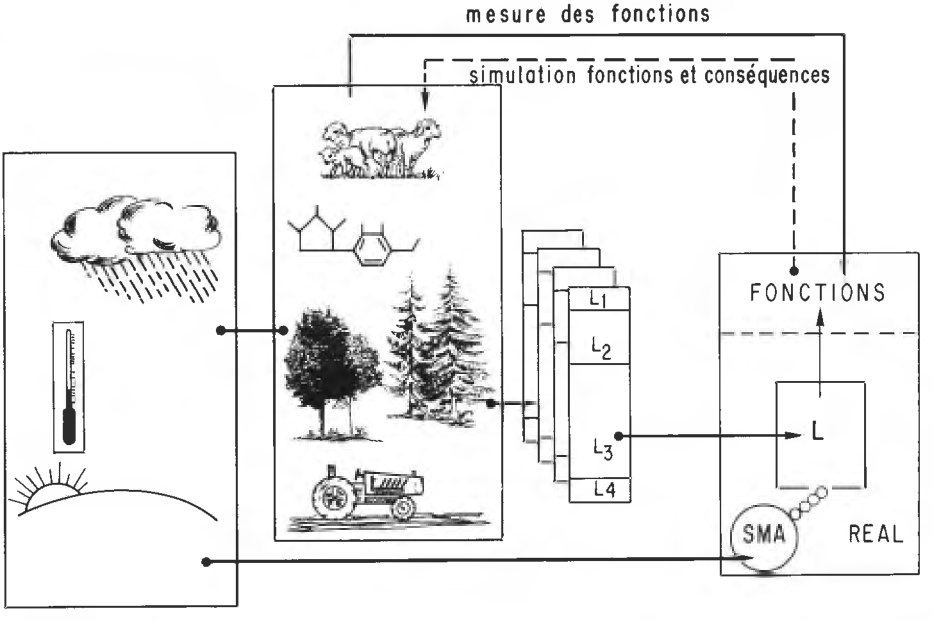

Figure 3. Transposability of REAL in various field conditions (grassland, forest, culture, *etc.*) under various pollutions leading to earthworm community states (populations L_1, L_2, ..., L_n), changing *versus* time (T). At each local condition the various earthworm functions depend on local variables: community state and activity of earthworms. The activity of earthworms depends of the Sub-Model of Activity (SMA) which depends mostly on temperature and soil moisture and on day-length (photoperiod). Most earthworm functions were estimated in fields and are simulated for fields. From [4].

4. Discussion

We are leaving the end of a historical period in science: the need to choose between increasing specialization and efficiency on the one hand, and fuzzy generalisations on the other. There is a conflict between the need to know precisely the effects of a pollutant under defined local conditions and the absence of a multivariate assessment into the variable global use of the same contaminant.

Due to the high diversity of pollutants, the great variety of their dispersal mechanisms and fate in the environment, the complexity and variability of soil components, including organisms, the use of organisms as bioindicators seems impossible or highly risky. This complexity must be first accepted and managed with its consequences: a bioindication could be hardly univocal and a bioindicator is mostly a bio-informant on the ecosystem states. This complexity must also be managed in grouping together all of the bio-information we collect about pollutants and ecosystems. This grouping must order the various elements of knowledge we have, and this ordination must allow the retrieval of those elements that are needed to solve each specific problem. Rather than to continue to make specialized studies independently and to exchange only some tiny parts of them as "results" we must absolutely group all our knowledge and share it. This is now possible.

The organization of knowledge could improve pollutant assessments and allow us to recognize the most vacant areas of knowledge, on which, therefore, we must focus our research efforts. Today, among soil animals, earthworms allow us a rather complete set of assessment approaches, with some drawbacks and a general need of validation and elaboration. This is only possible if we focus our research together, and if we open the access of the knowledge to nearly everybody.

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