Uptake kinetics of essential and non-essential metals by soil-invertebrates;
Dynamic Energy and Mass Budgets underlying metal uptake.

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1 PhD-Project; unravelling the link between environmental availability and bioavailability.

Many Dutch floodplain ecosystems deal with a high amount of heavy metals in the soil. It is likely that potential effects on biota can occur. Rijkswaterstaat initiated the question to derive a scientific based link between actual metal concentrations and effects in floodplain ecosystems; a pragmatic assessment tool to assess risks when environmental conditions are changing due to inundation or redevelopment of the floodplains. As demonstrated in many experiments, the impact of soil metal availability is hard to separate from effects of soil physico-chemical properties. The project is guided by two distinctive phases namely a physico-chemical based investigation and an ecotoxicological investigation. Metal distribution and speciation in terrestrial floodplain systems as a dynamic function in space and depth is investigated. For predicting adverse effects in biota accurately in a dynamic environment, it is necessary to develop procedures that take into account the mechanisms behind uptake and elimination processes. The aim of this research is to create more insight on metal uptake mechanisms, by matching chemical supply of soil to biological uptake and physiology of species. The results can assist the interpretation of metal uptake by biota under fluctuating conditions, which are characteristic in flood plain systems.

2 DEB in biological systems

The DEB-theory gives mechanistic rules that describe the uptake and use of energy and nutrients in organisms, which has consequences for physiological organisation throughout an organisms' life cycle, including toxic pressure. The model considers biota as an input-output system of energy. Food is input of energy, inside the organisms it is stored in energy reserves. Energy in the reserves is utilised for maintenance and growth (fraction K), development and reproduction (fraction 1-K). All processes have overhead costs. Maintenance is defined as the mean energy requirement of an organisms. These costs are species-specific, main factors are size and body temperature.

The Kappa-rule leads allocation of energy in the body. It makes growth and development parallel processes that interfere only indirectly. The use of reserves is partionable such that the dynamics are not affected. For example, if conditions are poor, the Kappa-system can block allocation of energy reserves to reproduction, while maintenance and growth continue to compete in the same way as before. Somatic maintenance has a higher priority than maturity costs.

Structural body mass and reserves are the state variables of the individual; they have a constant composition (strong homeostasis).The DEB-model distinguishes structural body mass from stored materials. Structural body mass is the total mass of body tissues that is essential for living, which costs energy. Stored materials are energy reserves and eggs.

In the metal accumulation studies this DEB-theory can assist in modelling uptake and use of essential metals (Chapter 4). Chapter 6 will be used to understand the uptake and effects of non-essential metals in organisms.

The metal research also deals with the impact of fluctuating external conditions (inundation) on metal uptake by soil organisms. I do not describe this aspect here, but will give a list of important (hypothesized) factors on the physiology and metal uptake of organisms:

- waterstress,
- decreasing food,
- oxygen reduction,
- (relative small) temperature decrease,
- different speciation of metals in the soil, thus different availability

When a certain level of toxic pressure for the organisms exists, it will decrease its somatic costs. We can think of decreasing activity of the organisms under these conditions. Lower metabolism, (maybe osmoses of essential metals over membrane will be influenced actively by biota), the maintenance costs will change from supply driven systems to demand driven systems.

Measurement of vitality/fitness of the organism is mostly done by measuring reproduction and population growth density. For the chosen earthworm species and isopod species the life-cycles are relatively long. Therefore, it is easier to investigate the healthy appearance of these species under certain circumstances. If we perform an inundation-experiment, we have to be aware of the fact that we do not change one environmental condition (as waterstress), but more interactive conditions (as described in the list above). We need a longer adaptation period before we can state what we measure and which causes give the possible adverse effect. The time of adaptation relies on what we are focusing on.

3 Metal uptake and elimination

Essential metals

Cu and Zn, both essential metals, are used by biota for the enzyme synthesis, DNA etc. Due to these physiological fates organisms are able to regulate Cu and Zn. These processes are not (directly) coupled towards energy budgets. Uptake of Cu and Zn can be both passively and actively by the organism. But it is more likely that there exist reserve-pools for these metals inside the organism, as the case of energy. Therefore, the uptake model of metals can be described as an assimilation model like DEB is. Metals are the input of the model, and will be stored in metal reserves. The use of reserves is partionable such that the dynamics are not affected. I state this, because organisms are able to deal for a certain time without the input of these essential metals. Inside the body the elements will be partitioned over the different reserve pools for the processes maintenance, growth, reproduction and development.
We should measure if there exists a constant ration of Zn/Cu in the reserves and in the structure. And how this reacts on fluctuating external concentrations, thus input. Most likely, we have to separate the possible uptake routes and investigate their relative contribution of uptake.

Non-essential metals

Non-essential metals differ from essential ones by the absence of regulated use. Uptake of non-essential metals occurs passively with the uptake of essential metals and via the macro-ion channels in the biological membrane. Therefore, non-essential metals are taken up in a similar way to essential metals. The DEB-theory can be used to specify the uptake and effects of the processes in a quantitative way. DEB allows us to a realistic incorporation of physiological interactions to the accumulation model. Parameter values of a "normal" accumulation model will be changed by xenobiotic elements.

4 Accumulation and effects

Organisms respond to exposure concentration. When organisms are exposed to high concentrations of essential metals, the organisms can control their internal level (until a certain exposure level). Non-essential metals cannot be regulated actively by biota. Consequently, the internal concentration of zinc and copper vary in a different way with soil concentrations up to a maximum value at which toxicity disrupts the pattern. The optimum internal concentration range in which the performance of the organisms is healthy, is named "the window of essentiality" in the toxicity. Exceeding or deficiency of essential metals, respectively, above or below the optimum "window of essentiality" leads to adverse effects. In concentrations exceeding the toxic level, the organisms possibility to regulate changes into an insufficient control of the flux by the animal, whereby a fast increase follows. A similar pattern is seen with non-essential metals, only here the window of essentiality is approximately zero (depending on the metal species). The net result of uptake and elimination is affected when xenobiotics are entering the body in a too large amount or when deficiency or toxic pressure due to a too low or high amount of essential metals is in the organisms' body. The parameter values of the one compartment model are changed, the Kappa cannot control the internal reserves anymore. Parameters versus these concentrations will probably give a linear relationship. Exposure for longer periods to high external concentrations, have influences like function losses, on the healthy appearance of biota. A totally different relationship between parameters versus concentrations will appear.

Elimination driven accumulation heights

The process which exists inside the organism is generalised in a formulae according to the "donor-control" principle, which states that external concentrations determine the uptake by an organism,

whereas internal concentrations conduct the elimination of a metal. In addition, the DEB-theory takes uptake from the food into account; because uptake from the environment concentration cannot be described in the same way as food.
Under optimal conditions, this can be simulated by a one-compartment accumulation curve, whereby the initial slope of the curve is characterised as the uptake. The plateau level is indicative of the internal steady state concentration. It is quite possible that the fugacity between the environment and the organism is not equal, because of kinetic processes (metabolism, degradation) in the organism. Steady state is the equilibrium which is found in practice: In = Out + pool (pool = metabolism, inert storage, e.g.).
The main assumptions of the one-compartment model are:
1 the supply capacity of the soil is endless
2 k_1 and k_2 are constant in time
3 no toxic effect in the animal occur
We know that in soil systems assumption 1 is a simplification of reality. From the DEB-theory we can learn that when some toxic stress exists, assumption 2 is not realistic anymore, even though effects are not seen yet. We can conclude that this one-compartment model only can describe our data as long as the exposure concentrations are not that high. We can state that we believe that accumulation height will be mostly influenced by elimination rates.
For energy it does not matter where in the gut uptake takes place, due to the fact that blood has a low capacity for energy but a high transport rate. (TJALLING). I believe that for metal uptake, this is not the case. The more food is intensively digested under gut conditions, the more mucus is mixed with the food. Food will fall into pieces, and metals will be less strongly bound towards the food. Affinity towards the membrane will increase, so more uptake possibilities. For internal partitioning of metals it may be the same as for energy; [transport rates do not have such large influences in shorter time periods. Later, metals will partition more over certain storage organs]. But the difference among uptake of energy from food and metals from food is the strong binding capacity of food for metals. Complete digestion will give similar results as energy uptake, but a digestion is never 100%. Another contradiction for metal uptake is that it is dependent on the Ca^{2+} concentration in the biota and in the environment. Uptake of metals goes for approximately 70% via Ca-channels. We have to deal with competing uptake mechanisms; how higher the ratio Ca/metal, the less metal will go inside the body passively. Maybe we need an additionally factor in the accumulation model, dealing with these concentrations.

5 Compartment modelling
On basis of the DEB-book, I chose to work with the one-compartment model. Multi-compartment models require a large number of parameters.

The feeding rate is proportional to the surface area of the organism and the food handling time and the digestion efficiency are independent of food density, is stated in the DEB-book. Although for metal uptake we deal with a passive uptake (assessed >70%) and an active uptake (< 30%) process, [which are lead by the relative contribution of each possible uptake route], the uptake rates are taken to be proportional to the concentration in the environment and/or the food uptake. As already suggested before, we maybe have to introduce a factor for Ca-concentrations. The formulae for uptake and elimination is:

$$(d/dt)c_V = (k_e /l)\,(c_d + Pd_X\, fc_X - P_{VW}c_V) - c_V\, (d/dt) \ln l^3$$

(equation 6.17 in DEB-book)
Explanation of the parameters:
Length of the organism = l (growth dilution incorporated)
Passive (constant) uptake = c_d
Scaled tissue concentration = c_V
Partition coefficient structure/total = P_{VW} (fat content is incorporated, also changing reserve density; while it is the partitioning between total body and structural body mass)
Uptake from food = $Pd_X\, fc_X$

Elimination rate from the body = k_e /l
Elimination rate (ratio of uptake rate from water to the elimination) = $P_{VW}c_V$
Elimination rate due to growth and reproduction = c_V (d/dt) ln l^3
We can now deal with:
- dilution by growth (juvenile or reproduction for adult)
- changes in lipid content

In DEB-context, the conservation law for energy allows us to take into account:
- toxicants affect energetics
- (cumulated) effect (no effects, on survival, on fitness/vitality, on reproduction and growth, receptor-mediated effect, effects of mixtures (MARTIJS))
- (metabolic transformations are not that relevant for metals)

These are extrapolations towards population densities. DEB conceives populations as a group of individuals. Reduction of food uptake has indirectly effect on reproduction and thus the population. The DEB model describes the routes that translate these effects into an effect on reproduction; allocation to reproduction depends on reserve density, which depends on feeding rate, which depends on body size, which depends on growth. Maintenance competes with growth for allocation, so effects on maintenance can be translated into effects on growth, and thus effects on reproduction. But for metal uptake these relationships will be more complicated due to the fact that metals can be taken up via the skin and via the food. These uptake routes are not directly related to each other. Less metals in the environment, can be compensated by lots of metals in the food (and the other way around). Troubles start if both metal sources have high concentrations of metals.

It is possible with use of the DEB-theory to obtain a wide range of experimental results from one simple experiment if we understand toxicity pattern. The effects of a particular combination of organisms and toxicant can be assessed by modelling.
Metal toxicity patterns depend on:
- temperature; because all rates depend on temperature. Physiology mechanisms increases a factor 3 with temperature increase of 10°C. Uptake kinetics are physiologically controlled and, therefore, increase for increasing temperature. For elimination this is quit similar, but less strong.
- pH; affects abundance and bioavailability of metals. Elimination rate is independent on external pH. (while biota maintain their pH on fixed levels – homeostasis). Uptake increases with lower pH, sensitivity as well.
- body size and reserves; uptake and elimination rates are inversely proportional to volumetric weight. Vitality is independent from body size (not from fat content)
- sensitivity of species; depends on which organ can it be stored, what is the target organ. As proposed in the DEB-theory, the uptake route has on longer time-scales no impact on the storage place. As the characterisation of toxic effects is built up in: uptake and elimination kinetics and the translation of internal concentrations into effects (critical body burden concept).