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Invertebrate Organisms as Biological Indicators of Heavy Metal Pollution

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ABSTRACT

Some species of invertebrate animals are known to be efficient accumulators of trace elements. Generally, metal accumulation by such organisms is based on efficient detoxification mechanisms, such as intracellular compartmentalization, or metal inactivation by binding to metallothioneins. Metal accumulators have often been used as accumulation indicators of environmental metal pollution. This means that, ideally, metal concentrations in the animal's body reflect quantitatively or semiquantitatively environmental pollution levels. In reality, however, many factors, such as the animal's weight and age, can disturb such quantitative relationships. These factors have, therefore, to be considered carefully before an invertebrate is utilized as accumulation indicator for metal pollution.

Apart from accumulation, many invertebrates exposed to elevated metal concentrations respond to this stress by metal-induced synthesis of metallothioneins. Additionally, metallothionein in metal-loaded organisms can be present in different isoforms that are specifically synthesized in response to different metals. These facts make metallothionein a potential biomarker for metal stress in invertebrates. One possibility may be to assess parameters of metallothionein synthesis at the molecular or biochemical level. Moreover, metallothionein isoform patterns could provide information on different isoforms synthesized in response to different metals or chemicals. In any case, however, care must be taken to consider intrinsic physiological parameters, such as nutritional or developmental factors, which could also interfere with metallothionein synthesis.

Index Entries: Invertebrates; heavy metal; metal accumulation; biological indicator; biomonitoring; biomarker; metallothionein.

INTRODUCTION

The process of metal accumulation by an invertebrate depends on a variety of intrinsic and extrinsic factors, but the functional anatomy of digestive organs and gill epithelia as well as the physiology of an organism play dominant roles in controlling accumulation processes. Thus it is not surprising that related species or taxonomic groups of invertebrates often share common accumulation strategies for essential or nonessential trace elements. Terrestrial gastropods, for instance, are known to be efficient accumulators of copper, cadmium, and lead (*1*). Similarly, some groups of crustaceans (*2*) or annelides (*3*) accumulate certain trace elements more efficiently than species of other invertebrate taxa, such as many insects (*4*).

Since their accumulation potential has been known, these animals have been utilized as biological indicators of environmental metal pollution (*5*). As the use of an invertebrate for biomonitoring in this case is based on the fact that accumulated metal concentrations in the animal's tissues reflect quantitatively or semiquantitatively the pollution level of the environment, such species have been called accumulation indicators. Terrestrial isopods and gastropods, for instance, have been used successfully as accumulation indicators of soil pollution by cadmiun, lead, copper, or zinc (*6–8*).

Apart from its accumulation capacity, each individual responds to metal stress by a series of definite physiological and biochemical reactions. In many metal-exposed invertebrates, for instance, the synthesis of metallothioneins is induced. Metallothioneins are low-mol-wt, cysteine-rich proteins displaying a high affinity toward certain trace metal ions such as copper, cadmium, and zinc (*9*). The role that these proteins play in cellular metabolism is rather complicated, but one of their dominant functions is related to the protection of cells and organisms against toxic metal stress (*1*). Because of the inducibility by metals, metallothioneins can be used as indicators of pollution stress to which an invertebrate has been or is actually exposed. Thus under such circumstances metallothioneins of invertebrates can serve as biomarkers in environmental monitoring (*10*).

INVERTEBRATES AS ACCUMULATION INDICATORS

Generally, the elevated capacity of metal accumulation in an invertebrate species is based on detoxification mechanisms, which may confer to the animal an increased resistance against accumulated toxic elements. In particular, two mechanisms are of major importance: Intracellular compartmentalization of metals by vesicular inclusion and chemical inactivation of metals by binding to metallothioneins (*1*). An example of the first mechanism is provided by isopods for lead, cadmium, and copper (*2*), whereas cadmium in gastropods is detoxified according to the second mechanism by binding the metal to metallothioneins (*11*).

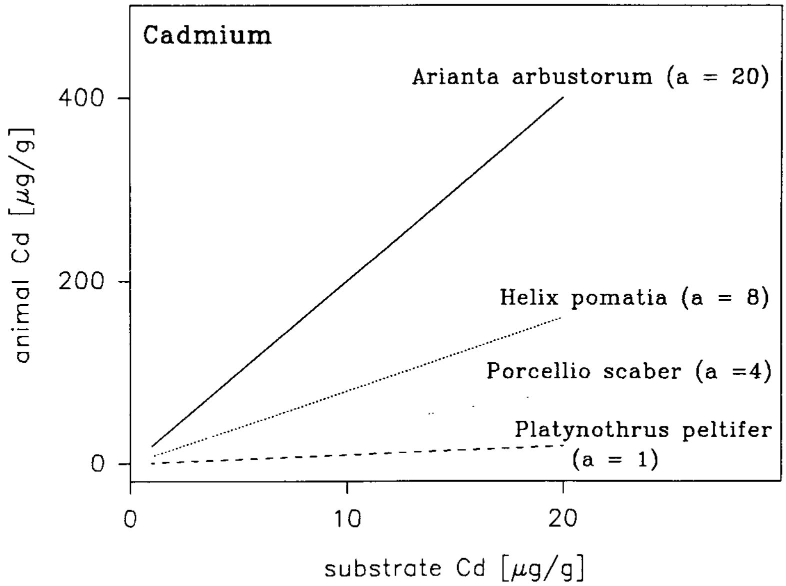

Fig. 1. Linear relationships of biological concentration factors ($a \cdot x$) for cadmium in different terrestrial invertebrates according to Eq. (1). All data have been taken from Dallinger (*1*). Although *A. arbustorum, H. pomatia,* and *P. scaber* have already been used successfully in biomonitoring, the linear relationship for the oribatid mite *P. peltifer* ($a = 1$) is speculative.

In both cases, accumulated metals are concentrated predominantly in the invertebrate's midgut gland and stored over extended periods of time. Moreover, the metals concentrated in the animal's tissue reflect quantitatively the metal concentration of the substrate on which (or in which) individuals live. Ideally, this quantitative relationship can be described by the linear equation:

$$y = a \cdot x + b \tag{1}$$

where y is metal concentration in the animal's body, x is metal concentration in the substrate, and a is biological concentration factor.

Obviously, the biological concentration factor a in Eq. (1) is the link between metal concentrations in the animal's body and those of the substrate on which or in which an individual lives. In any case, the biological concentration factor defines by how much an invertebrate concentrates a given metal in relation to environmental concentrations. For purposes of biomonitoring it is crucial that the biological concentration factor a remains constant over a broad range of metal concentration in the environment (*see* Fig. 1).

In reality, many factors can interfere with such relationships. For instance, the biological concentration factor a for a given metal in an invertebrate depends on the animal's body weight and its age (*7*); moreover, the presence of additional elements in the substrate may falsify the results. In

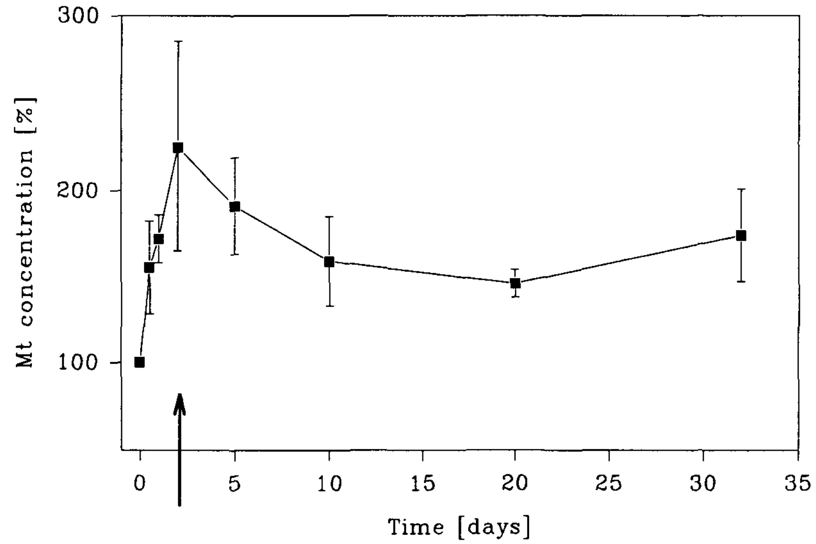

Fig. 2. Relative metallothionein (Mt) concentration (expressed as percentage of controls: *y* axis) in cadmium-exposed Roman snails during a 2 d metal exposure (time: *x* axis) via the food. At the end of the second day (arrow), cadmium exposure was stopped, and animals were fed a noncontaminated diet until the end of the experiment. Data have been taken from Dallinger (*1*).

the presence of elevated amounts of zinc in the soil, for instance, earthworms fail to fit into a linear relationship as described above (*3*). For the practice of biomonitoring this means that disturbing factors must be considered carefully before an invertebrate can be used as an accumulation indicator.

METALLOTHIONEINS AS BIOMARKERS FOR METAL STRESS IN INVERTEBRATES

In gastropods, cadmium is accumulated and concentrated predominantly in the midgut gland of these animals by binding the metal to metallothioneins. Recently, for instance, a cadmium-binding metallothionein isoform has been purified from the midgut gland of the terrestrial snail *Helix pomatia*, and its primary structure has been elucidated (*12*). As shown by our previous studies, cadmium is a potent inducer of metallothionein synthesis, at least in terrestrial gastropods (*1*): As seen in Fig. 2, cadmium administration via the food-induced metallothionein synthesis in the midgut gland of exposed Roman snails within only a few hours. Apart from this, metallothionein is present in several metal-specific isoforms in the tissues of these animals (*11*). All these facts make metallothionein a potential biomarker for biomonitoring purposes (*13*), using parameters of protein synthesis in suitable assays to detect nonlethal or sublethal metal stress in invertebrates (*14*).

One possibility to utilize metallothionein as biomarker in the sense mentioned above might be to assess metallothionein synthesis at the molecular level, adopting polymerase chain reaction (PCR) amplification of metal-induced metallothionein mRNA. In this way, the efficiency of metallothionein synthesis could be tested in a screening assay using different metals in laboratory experiments and in the field. Another possibility would be to measure metallothionein concentrations directly (*see* Fig. 2), using recently described standard methods (*15*). Eventually, isoform patterns of metallothionein could also provide information on specifically synthesized isoforms induced because of exposure to different metals or chemicals. In any case, however, care must be taken to avoid nonspecific influences derived from seasonal variation or nutritional factors, which could also affect metallothionein synthesis (*14*).

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