Research Signpost
37/661 (2), Fort P.O., Trivandrum-695 023, Kerala, India

Recent Res. Devel. Entomol., 4(2003): 1-17 ISBN: 81-271-0013-7

Ecological consequences of bioavailability of metals and metalloids in insects

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Description of general physical properties of the metals and metalloids

The chemical definition of a metal is an element with a lustrous appearance, that is solid at room temperature (except mercury), a good conductor of heat and electricity, malleable, ductile, and one that generally forms positive ions in solution. To a biologist, only the latter of these criteria is relevant, and a more specific definition is required. Eighty-seven of the elements on the periodic table are considered metals or metalloids (elements which act like metals). Of these, many are important pollutants but do not have all of the properties of a metal (e.g. selenium), while others do not warrant discussion with other metals as pollutants since they are major nutrients (e.g. calcium). Categorization based upon relative density greater than five, or an atomic weight above that of sodium, also

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includes or excludes elements commonly associated with pollution (1).

From a toxicological perspective, a categorization put forth by Martin and Coughtrey (2) is useful for its applicability to the field. These authors suggested that a metal is an element with permanence, toxicity, and a long residence time in the environment. These are some of the same characteristics that make metals and metalloids difficult for organisms to regulate. In general, these elements are toxic at relatively low levels to arthropods and many other organisms. Because metals and metalloids are such a large and diverse group of elements it is not surprising that the mode of action and concentrations resulting in toxicity to arthropods are variable. In fact, mode of action and concentrations resulting in toxicity vary not only by material, form of the material, and animal species, but even by the particular life stage of the organism tested (3).

Collectively, these effects often result in impaired growth and development and the disruption of reproduction. Therefore, abundance and species diversity are usually diminished in areas where metal pollution is present.

Common sources of ecosystem pollution

The main sources of metal contamination have been examined in detail by Hopkin (1). Briefly, natural sources include aerial fallout of dust particles emitted from volcanic activity and weathering of geological deposits. Anthropogenic sources include industry, mining, smelting, combustion of fossil fuels, and agriculture. In an industrial society, metals are some of the most commonly used raw materials. Consequently, wastewater runoff from mining, metal refining, chrome plating, sewage sludge, and other anthropogenic sources contain high levels of metals that pollute water and soil. Additionally, exhaust from combustion engines, as well as emissions from energy and fuel production, smelters, and foundries contain metals as airborne particulates. Airborne particulates containing metals often precipitate onto the surface of plants, damaging plant photosynthetic systems and resulting in altered plant chemistry and nutrition for herbivores (4). Contamination of soils also allows for plant uptake of many metals making these elements available to herbivores.

A survey of the recent literature shows that all of these sources are indeed still active producers of metal contamination. Several important industrial sources of pollution were identified in studies by Glowacka et al. (5) and Vajpayee (6) who examined chromium contamination as a result of metallurgical, dye and pigment, plating and textile industries. Contamination from mining activities was reported by Croteau et al. (7) and Naqvi and Rizvi (8). Cain et al. (9) documented the effects of acid mine drainage, and Schultheis et al. (10) studied the effects of the castings from a pyrite mine closed over 50 years ago. Zvereva and Kozlov (11) recently reported the effects of atmospheric deposition from a nickel-copper smelter. The relative importance of combustion of fossil fuels has been shown (12), in addition to the devastating effects on animals of coal fly ash receiving ponds at power generating facilities (13, 14). Winder et al. (15) and Merrington et al. (16) studied the effects of elevated metal concentrations as a result of fertilizer application for agriculture, and Fan et al. (13), Hamilton (12), Sappington (14), and Thomas et al. (17) tackled the problem of selenium contamination due to agricultural drainage. Thus the sources of metal contamination, whether caused by current anthropogenic activities or resulting from much earlier events, continue to exert substantial effects on a wide variety of ecosystems.

History and patterns of release

Problems with pollution are not new; air and soil contamination have been reported for thousands of years. Over 2000 years ago the Roman poet, Horace, complained about soot damaging the walls of temples. Two millennia ago the Greeks and Romans generated enough lead pollution to leave a record of residues in the glaciers in Greenland (18). However, the global problem has become substantially worse since the Industrial Revolution with the large-scale production and transport of many toxic materials. Since the Industrial Revolution, most countries have evolved through a predictable pattern. They generally start with a period of intense industrialization, where the primary goal is to raise the standard of living for the population. During this period the environmental and even health effects of pollutants are generally considered secondary concerns, positioned well behind the necessity of feeding the local population. A recent example was described in northern India by Rai and Sinha (19) and Rai et al. (20), where debilitating levels of chromium (135 μl/l) and copper (1590 μl/l) were present in aquaculture ponds, and even higher levels in the aquatic plants (lead at 723 μg/g, chromium at 517 μg/g, and iron at 2490 μg/g dry weight). Both the fish and the plants continue to be harvested for human consumption.

As countries become more affluent, the desire for improving environmental quality increases. However, even in situations where most sources of pollution have been largely eliminated, a 'legacy' of contamination may still exist. This is exemplified by streams in central California (U.S.A.) where a gold rush over a century ago left entire watersheds contaminated with mercury (21). Both the mercury mines and the sites where mercury was used to extract gold ore are contaminated. Similarly, in central Mexico, an estimated 45,000 tons of mercury were shipped from Spain to extract gold and silver, and much of this remains as a major source of contamination (22). History is not the only factor contributing to the lingering problem; nationalistic concerns, economic costs of less polluting technologies, and long standing patterns of industrial production work to impede changes that can reduce contaminants. It is evident that the problem of metal pollution is likely to continue for the foreseeable future.

The problem is global

Virtually no region on earth is free of significant metal or metalloid pollution. Hamilton (12) reported selenium at high concentrations in marine life in Norway, Finland, and the Faroe Islands. He also listed sewage sludge in western Europe at 5 μg/g, drinking water in Italy 9 μg/l, and saline lakes in Pakistan at 297-2100 μg/l. The many contaminated sites in U.S.A. have been reviewed by Skorupa (23). Antarctica has elevated concentrations of many metals incorporated in ice and snow deposits from atmospheric deposition (24) and by point source pollution generation (25).

The 'Economics of Industrial Pollution Control Research Project' (26) has developed a database for estimating the amount of industrial pollution for any country. This system, called the Industrial Pollution Projection System (IPPS), estimates a nation's pollution output based on the number of workers employed in selected, polluting industries. Even a cursory review of this very large database indicates that every continent and essentially every nation currently have multiple industries with significant potential for metal and metalloid pollution. Thus, even if an individual country eliminates most point sources of metal pollution, the problem will persist through atmospheric and aquatic transport across

political boundaries, by trade activity, and from legacy pollution. While individual countries can and should attempt to minimize such pollution, the problem will ultimately need to be handled as a global issue. The international nature of this problem was recently recognized by the United Nation's 1998 Aarhus Protocol on Heavy Metals to the 1979 Convention on Long-Range Transboundary Air Pollution on Heavy Metals (27). However, at the time this chapter was written, only 13 of 36 signatory countries had ratified the agreement.

Arthropods and measurement of ecosystem scale effects of metal pollution

While there is an abundance of information on the global scope of metal pollution, substantial published data on organismal and population level effects have only recently become available, particularly for terrestrial organisms near the bottom of the food web. This chapter therefore will focus on the impact of metal and metalloid pollution on insects and related arthropods. For effects of metal pollution on higher level organisms such as birds, fish and mammals, the reader is referred to (28, 29, and references contained therein).

Arthropods are present in high densities and fill many niches in almost every ecosystem and are therefore responsible for a significant amount of energy transfer from primary producers to carnivores (30). Our perception of limitless and ubiquitous insect pests often belies their importance in general ecosystem dynamics, and allows their roles as detritivores to be overlooked when considering the impact of a toxicant on an ecosystem. Indeed, insects may be some of the first animals to re-colonize a contaminated site, but which insects, their functions, and their ultimate abundance offers insight into the true impact of the pollutant (10).

Choosing an insect for testing may not always be a simple process. Van der Geest et al. (31) examined the sensitivity of native mayfly nymphs and found them to be far more sensitive (two orders of magnitude) to copper contamination than 'standard' test species (*Chironomus* spp. and *Gammarus* spp.). Mayfly nymphs were also the first to disappear and the last to reappear in the contaminated site, even when pollution levels were low. Thus, standard test insects may not be relevant to every ecosystem. While it can be useful to have standardized organisms for comparison and efficiency of testing, this study shows that disregarding the effects of a contaminant on native populations can seriously underestimate the ecological significance of a pollution event.

Schultheis et al. (10) also examined benthic invertebrates, but with a focus on how contamination affects the structure and function of an aquatic community. Taxonomic richness as well as leaf decomposition rates declined at a copper-contaminated site in Virginia as compared to a control location. Interestingly, the authors continued the study after the remediation of the contaminated site (below known chronic levels) and found that while a recovery of taxonomic richness and structure occurred, there was not a recovery of leaf decomposition rates even six weeks after the apparent recovery was complete. While the resurgence of species richness is encouraging, the impaired function of the community is of concern given that species richness is used so frequently as an indicator of community health. Therefore, some measure of the functional response of a community may be needed to completely assess either the recovery of, or the damage at, a contaminated site.

Another potential measure of function with an ecosystem is the host suppression rate of naturally occurring arthropod control agents. Zvereva and Kozlov (11) studied effects of copper and nickel air pollution on natural enemies of the willow-feeding leaf beetle (*Melasoma lapponica*). They reported that densities of the leaf beetles were higher at copper-nickel contaminated sites, but the total mortality caused by natural enemies at clean sites was higher than at polluted sites. In fact their data documented that the decrease in predation materially contributed to increased leaf beetle density at the polluted sites. Thus, in this case, the common practice of sampling only the insect herbivore populations would not provide an accurate estimate of ecosystem effects.

Acquisition of metals and metalloids by arthropods

There are two major routes of metal ion uptake in aquatic invertebrates, the first is through respiration, i.e. the water in which they live, and the second is through ingestion (32). It is difficult to generalize about which route is more important since uptake varies according to the type of metal, the species, the developmental stage of the invertebrate, and even the ambient physicochemical conditions (33, 34, 35, 36, 37).

The importance of oral exposure becomes apparent when one considers the amount of material ingested by each insect. Ankersmit et al. (38) estimated that to obtain a larval weight necessary for pupation (28 mg), the syrphid *Episyrphus balteatus* requires 145-150 third instar aphids at 20 degrees Celsius. The addition of the small amounts of metal in each aphid would be magnified many times if the metal was biologically available and not egested by the syrphid larva. This case is conservative considering the nutrient-rich insectivorous diet; herbivorous insects have to process many times this quantity of plant material in order to obtain required nutrients, and could subsequently be exposed to even higher levels of metals. For example, Naqvi and Rizvi (8) tested the accumulation of chromium and copper in alligator weed. They found that both metals accumulated in the plant, with concentrations in the roots ranging from 10 to 200 times the concentration in the sediment/soil. Aquaculture ponds in India with high metal contamination in the water (~15 to 100 ppm) have even higher levels of chromium, lead, copper, and iron (650 μg/g, 723 μg/g, 1590 μg/g, 2490 μg/g dry weight respectively) in the aquatic vegetation, which is destined for human consumption (19, 20).

Fan et al. (13) provide an excellent example of the complexity of metal acquisition by insects in their study of selenium biological transformations and subsequent pathway through the food chain. Selenium has several different oxidation states including selenate (Se+6), selenite (Se+4), elemental Se (Se0), and selenides or organic forms of Se (Se+2). Although Se causes toxicity at high concentrations, it is an essential trace nutrient important to humans and most other animals as an antioxidant (39). In most cases, sodium selenate is transported via agricultural irrigation water, then transformed within algae and plants to the organic forms (selenomethionine and selenocysteine)(40). It is important to note that acute toxicity from selenium associated with direct water exposure is not the cause of the ecological problem posed by selenium (41, 42), but rather chronic toxicity resulting from dietary selenium uptake and foodchain transfer. Fan et al. (13) found an average bio-magnification of 1400 times from water to microphytes and a further average bio-magnification of 1.9 times from microphytes to macroinvertebrates. This suggests that even though there are many water-soluble forms of selenium, the exposure to a metal through contact with water may not be insignificant, but is substantially less than

the potential levels that can be attained through oral exposure.

A study by Munger and Hare (43) provides additional evidence for the importance of oral uptake of contaminants. They examined the relative importance of water and food as sources of cadmium (Cd) to a predatory insect using a three-link food chain. This food chain was comprised of algae (*Selenastrum capricornutum*), a crustacean (*Ceriodaphnia dubia*), and the larval stage of the predatory insect *Chaoborous punctipennis* (Diptera). The authors observed that predatory larvae exposed to Cd in food alone did not contain significantly different levels of Cd than those exposed to contaminated food and water, indicating that the water exposure was negligible. They also reported preliminary data suggesting the same relationship was present in the crustacean prey of the *Chaoborus* spp. used in the experiment.

Filter feeding aquatic insects are also at risk from metal contamination, as their diet contains both microorganisms with bio-available metals in addition to insoluble metal particles. This highlights an important consideration regarding metal detection methodology. While the typical strategy of atomic absorption spectrophotometry (digestion, incineration) of the entire insect is often desirable or necessary due to their small size, studies have suggested this technique can overestimate concentrations biologically available to the test insect because substantial amounts of Al, Fe, and Pb can be adsorbed to external body surfaces (44, 34, 45) or stored as one of four types of sequestered metal granules in the digestive systems of terrestrial insects (46, 47, 48). To eliminate this concern Cain et al. (9) performed an analysis of trichopteran cytosol to measure biologically available levels of metals in these filter-feeding insects. They were able to document elevated cadmium, copper, lead, and zinc concentrations in the cytosol of Trichoptera living 120 km downstream of a contamination source.

In a terrestrial setting, insects have to acquire their nutrients, minerals and trace elements from food. Unwanted and toxic ions can readily cross the midgut epithelium and enter the hemolymph (49). Most literature supports the concept that the uptake of metals by terrestrial invertebrates is not under the control of the organism, but depends predominantly on the concentration in the food (50, but see 51). Indeed, Crawford et al. (52) found that grasshoppers fed on Cu and Cd-contaminated maize accumulated cadmium in direct relation to the amount ingested. While copper was also accumulated, the grasshoppers were able to excrete excess copper above a threshold level. Glowacka et al. (5) found metals accumulating in psyllids exposed to plants in a polluted area containing Al, Fe, Zn, Cu, Cd, Mn, Ni, and Hg. Similarly, Davison et al. (53) analyzed four insect orders (Coleoptera, Diptera, Hymenoptera, Hemiptera) for metal content. Although different responses were shown by each order to the metals, significantly higher levels in the hemipterans led the authors to conclude that plants can be seen as the most important route for the transfer of heavy metals originating in the soil into higher trophic levels.

Unfortunately, only adults were analyzed, and the entire body was ground up for analysis, which translates to uncertainty regarding the biologically available levels of metals in each order depending on their respective methods of detoxification, sequestration, or excretion. Another study examining relative toxicological responses across taxonomic categories was performed by Boyd and Wall (54). In this study, four different predator species (2 arachnids, 1 mantid, and 1 lacewing) were fed prey containing high levels of nickel. They found that while three of the species were not affected, one of the spider genera had a significant decrease in survival. Finally, Merrington et al. (16) followed Cd and Zn from fertilizer applications, through wheat

plants to aphids, and then to their lacewing predators. They found that the aphids accumulated concentrations of Cd and Zn some 24 and 140 times greater, respectively, than the concentrations in the fertilized soil on which the wheat plants were grown. However, they found that the predatory lacewings were not accumulating Cd or Zn through the aphid diet any differently than the controls, with the authors speculating that this was due to the piercing and sucking method of feeding by the predator and the location of contaminants in the body of the prey.

General physiological responses of invertebrates to metals

There are two ways in which invertebrates respond to an exposure to metal contamination; regulation or accumulation (32). These two strategies are not always mutually exclusive. Laskowski (55) suggested that terrestrial animals might be able to regulate physiologically essential metals, but not xenobiotics. Our review of the literature suggests that herbivorous arthropods, perhaps due to their short lifespan, have adopted an accumulation strategy for nearly all metals. Certainly, accumulation does not imply the absence of any sort of regulation, but rather a combination of excretion and sequestration, which still results in a net accumulation of metal in the insect. Among the invertebrates, whole-body regulation has been shown only with zinc and copper in decapod crustaceans (1, 56) and involves homeostatic body concentrations of the metal even upon exposure to increasing concentrations. Some insects practice a form of 'semi-regulation' whereby trace metals are excreted at each molt or during the pupal stage with each cast exuvium (57, 51, 58). Other species use a combination of regulatory strategies. In one unusual case, Glowacka et al. (5) reported that in psyllids, the exuvia were important for elimination of Al, Ni, and Mn, larval wax was an important route for the elimination of Al, Cu, and Ni, while large quantities of Al, Fe, Cu, Mn, and Cd were eliminated in honeydew.

Some insects have storage techniques that help minimize the bioavailability of metal in their bodies. Fangmaier and Steubing (59) reported that Pb was bound to calcium layers in the exoskeleton. However, accumulation in the exoskeleton is by no means the rule; storage in the epithelium of the gut or Malpighian tubules as granules has been reported in Dictyoptera (60), Lepidoptera (61), Diptera (62), and in solution on metal binding proteins (metallothioneins) in Plecoptera (63), and in Diptera (57, 62, 64). Witzel (3) reported two kinds of storage cells among the midgut epithelium in isopods. These include 'b' cells that secrete digestive enzymes and metal granules into the hepatopancreas to be excreted, and 's' cells that store the granules permanently. All of these storage methods essentially perform the same function by rendering the metals biologically unavailable, and hence non-toxic to the arthropod. These systems can certainly be overwhelmed, and symptoms of metal toxicity will occur at elevated concentrations.

At the opposite end of the spectrum there is good evidence that even very low levels of some metals can have major impacts on insect physiology. A detailed study of the effects of selected metals was performed by Bischof (65) who examined the effects of Cd and Zn on parasitized *Lymantria dispar*. She found that Cd and Zn caused a significant decrease in the concentrations of the major storage compounds (trehalose, glycogen, and hemolymph lipids). Not surprisingly, these decreases affected not only the host but for the parasite as well. Ortel (66) performed a similar study, investigating concentrations of proteins vs. amino acids in the hemolymph of *Lymantria dispar* exposed to very low levels of metals (Cu, Pb, Zn, Cd) that did not cause decreases in survivorship (at the no

observable effect level). She found a decrease in the concentration of hemolymph proteins, and an increase in the concentration of amino acids in the hemolymph. This lends support to the hypothesis that the presence of metals disrupts protein synthesis. This disruption would occur in the fat body, the central organ of intermediary metabolism, which regulates hemolymph amino acid composition and protein metabolism. Both the Bischof and Ortel findings occurred at metal concentrations that would be considered safe, because no mortality was observed at the tested levels. These physiological and nutritional changes have interesting implications for the ecology of host-parasite interactions. Nonetheless, putatively safe levels for metal exposure should not be based solely on developmental or survivorship data.

Toxicity and consequences of exposure to cadmium, copper, zinc, and lead

These four compounds are often grouped together due to their frequent co-occurrence as contaminants in the environment (51). They are often found in association due to the output from smelters and the application of sewage sludge as fertilizer. Of the four, cadmium is often the most toxic to insects, although it is not usually as abundant as the other three metals in most contaminated environments (7, 51, 5, 67). The other three elements may vary in relative toxicity depending upon the test species. For example, as discussed in the previous section, the Bischof (65) and Ortel (66) studies showed substantial effects at very low levels of Cd, Cu, Pb, and Zn. In contrast, Crawford et al. (68) studied the effects of elevated levels of Cu and Cd on the performance of aphids and found that, at the levels used, the metals had few deleterious effects on aphid growth, reproduction, or development. Similarly, Bruus Pedersen (69) found no effects from elevated copper using the Collembolan, *Folsomia candida*. Croteau et al. (7) even studied the potential for using a metal tolerant species (*Chaoborus* spp.) as a biological indicator of free zinc, copper and cadmium ions in acidic metal-contaminated lakes. The authors required a metal tolerant species because biological indicators needed to be tolerant to pollution since the insects were intended as an indicator of ion concentration, not the effects on biota. They found that Cd concentrations in the insect could be used to indicate biologically available Cd ions in the water. However, copper and zinc were too efficiently regulated by this species to be able to predict biologically available levels of these two metals in the water.

Environmental factors may also play a role in the relative toxicity of these four metals. Sandifer and Hopkin (67) compared the toxicity of Cd, Cu, Pb, and Zn at 15 and 20° C. They found that the toxicity of Cd, Cu, and Zn were not significantly different at the two temperatures. However, lead was twice as toxic at 15° C than it was at 20° C, although it was still the least toxic of the four metals against *Folsomia candida* (Collembola).

Relative toxicity is not always the most important factor in determining which of these metals will cause toxicity in a particular environment. Fountain and Hopkin (51) calculated a relative toxicity factor that took into account the concentrations of these four metals in the soil and the relative toxicity by oral exposure of each metal. They predicted that zinc, although it was the least toxic of these four metals to soil arthropods, would likely cause the greatest impact because it had the highest concentration in the soil at their study site.

While it is possible to segregate these four metals according to toxicity, they may also be categorized by where they are found within the body of the insect, and how they are regulated. Cain et al. (9) documented the partitioning of these metals between cytosolic and noncytosolic (particulate) forms within the body of Trichopteran larvae (*Hydropsyche californica*) in a stream contaminated by mining activity. They reported that 50 to 100% of the Cd, Cu, and Zn was located in the cytosol of the larvae, while 80 to 92% of the Pb was found in a particulate form. In an earlier study with similar results (70) these authors also found that the Cd and Cu in the cytosol varied with the concentration in the river, while the zinc in the cytosol did not. This implies the regulation of the zinc by the Trichopteran larvae, and the lack of regulation of the Cd and Cu. Additionally, Crawford et al. (52) studied locusts fed on Cd and Cu contaminated maize and found that the locusts were able to regulate copper levels at a threshold body content. In contrast, even though cadmium was egested, body content increased with exposure, indicating an inability to regulate Cd. As with the relative toxicity of these four metals, in vivo regulation is species specific and at least partially dependent upon environmental factors.

Toxicity and consequences of exposure to hexavalent chromium

Hexavalent chromium is one of the most common contact sensitizers in industrialized countries and is associated with numerous materials and industrial processes, including chrome plating baths, chrome colors and dyes, cement, tanning agents, wood preservatives, anticorrosive agents, welding fumes, lubricating oils and greases, cleaning materials, and textile production. Due to the past and present use of chromium in so many industries it is not an uncommon pollutant of ground water in many countries. Chromium occurs in several oxidation states, with the two major oxidation states trivalent chromium (Cr III) and hexavalent chromium (Cr VI) behaving very differently in the environment. Chromium III can form insoluble precipitates and behaves as a hard Lewis acid (71) while Cr VI is highly soluble. Public concern is related mainly to Cr VI since it has been found dissolved in drinking water and toxicity is associated with the oxidation of intracellular compounds (72).

Not surprisingly, chromium toxicity varies with species. For example, Canivet et al. (73) tested the toxicity of hexavalent chromium to larvae of two insect species, three crustaceans, and a snail. LC_{50}s were determined over 96 and 240-hr periods; toxicity increased with longer exposure times. Chromium was more toxic for crustaceans than either of the insects or the snail in the short duration test. However, in the longer duration tests, the insects (Ephemeroptera) were 9 to 26 times more sensitive. These results indicate that caution is required when setting water quality standards with short-term toxicity tests carried out with laboratory organisms.

Leslie et al. (74) studied *Hydropsyche pellucidula* (Trichoptera) exposed to chromium contamination in their natural environment (total chromium concentration including Cr VI and Cr III of 922 ± 102 μl/L). The anal papillae of immatures were discolored when exposed to high concentrations of chromium. The anal papillae are ion-exchange organs that are responsible for taking up ions from the water ensuring the hypertonicity of the insect's body fluids with respect to the surrounding aquatic environment. Papillae damage is typically accompanied by reduced fitness of affected individuals (75). Leslie et al. (74) expected that Cr VI would especially affect these

biological membranes since Cr VI is reduced to Cr III after absorption. Trivalent chromium has the ability to bond to biological macromolecules and would be difficult to excrete, accumulating in the organism. In this study, population level effects were documented, and the Cr was believed responsible for 1) the absence of life in the tributary at the source of the contamination, 2) the reduction in species diversity at the downstream site, and 3) the bioaccumulation and physical abnormalities in many surviving caddisfly larvae.

In an effort to examine how terrestrial detritivores might respond to Cr VI contamination, we designed an experiment testing the effects of Cr VI on the development time of *Megaselia scalaris* (Loew.) (Trumble and Jensen, unpublished). This dipteran is a small yellowish-brown phorid that was selected as a model insect for this study because it is a scavenger of nearly cosmopolitan distribution, and likely to feed on decaying material contaminated with many different pollutants, including Cr VI. Larvae of this species have been reported developing on a wide variety of host materials including decomposing meat, insects, and plant material (76). Using females from a lab colony, we synchronized oviposition and placed the eggs on commercial *Drosophila* diet prepared with six concentrations of Cr VI ranging from 0 to 1000 µg/g. Eight replicate Petri dishes containing 20 larvae each were tested for each concentration. The larvae and pupae were monitored daily, and the adult emergence date and sex were recorded for each individual. Figure 1 shows the number of days required from oviposition to adult emergence at each treatment. There was a significant difference in emergence time with increasing concentration of Cr VI in the diet (ANOVA $p=0.0001$), but no significant differences in emergence time between the sexes.

Our results show that *M. scalaris* would exhibit significant developmental delays at a Cr VI concentration as low as 0.5 μg/g. This delay in development time would decrease the intrinsic rate of population increase and expose the remaining larvae to additional

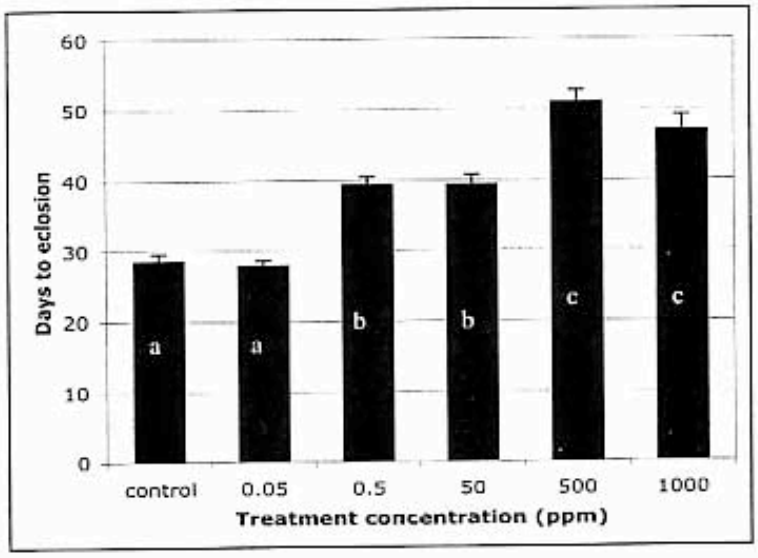

Figure 1. Effect of various Cr VI concentrations on adult eclosion times of *Megaselia scalaris*. Bars with different letters have significantly different ($P<0.05$) eclosion times (ANOVA, Fisher's protected LSD test).

mortality from environmental factors or predators. The increase in developmental time (54% at 500 μg/g as compared to the control) could also impact population survival, because theavailability of food sources for the larvae are often temporary (76), and extending developmental times could exceed the suitability of the food supply.

Toxicity and consequences of exposure to mercury

Mercury is considered one of the most hazardous compounds commonly released into the environment (77). It occurs naturally in low concentrations in the soil (<0.02 to 0.05 μg/g), but may exceed 1μg/g in agricultural soils (78). This metal has been used widely as a fungicidal seed coating, as well as for several industrial and military purposes. Major sources include mining, smelting, combustion of fossil fuels, and agriculture (79). Like other metals, Hg accumulates in soil due to an affinity for clay particles and organic humus.

Several studies have shown that Hg is quite toxic to insects. Zhang et al. (80) found that Hg bioaccumulated in the German cockroach (*Blatella germanica*) with chronic exposure. Profound and irreversible pathological abnormalities occurred in the ovary, testis, alimentary canal and the fat body. Even the symbiotic relationship between bacteria and fat body cells was negatively affected. Acridid nymphs developing on Hg-contaminated diet had prolonged nymphal stage duration, lower adult weight, and shorter adult lifespan than control insects that received no Hg (78). The egg-laying female acridids of *Aiolopus thalassinus* were unable to distinguish between treated and untreated substrate, even though the number of hatching nymphs decreased with increasing mercury chloride concentration. Interestingly, female *Acrottus partruelis* produced more eggs when exposed to sublethal doses, but an extended larval period resulted, and only 30% of the nymphs reached the adult stage.

Biomagnification of mercury was reported by Nuorteva et al. (81) who fed contaminated flies (40 μg/g) to tenebrionid larvae, and found that the beetle larvae contained more than 200 μg/g of mercury after four months. Similarly, mercury accumulation was also shown to occur in aphids to levels high enough to be toxic to their natural enemies (82). However, acute effects of mercury toxicity can occur at much lower levels (1 to 10μg/g dry wt; 11).

Like many other metals, the chemical form of Hg determines its availability to primary consumers. Bacteria readily methylate the inorganic form as a potential detoxifying mechanism, since the methylated form is more soluble and may be easier to eliminate (83). Unfortunately the methylated form is highly toxic to higher organisms. Inorganic Hg has been reported to produce harmful effects at 5 µg/l in a culture medium, while organomercury compounds can exert the same effect at concentrations 10 times lower than this (84). Lodenius (85) reported that sarcosaprophagous dipteran larvae assimilate more mercury from their food when the mercury is methylated, compared to the non-methylated form. Saouter et al. (86, 87) also noted that methyl mercury accumulation was much more rapid in the mayfly than the inorganic form. It is clear that mercury, and especially the methylated organic form of mercury, is a very toxic metal to insects. The bioaccumulation of mercury in insects has the potential for a substantial impact on vertebrate predators that feed on insects. This accumulation is cause for further concern since humans are exposed to these same predators through fishing and hunting for sport or sustenance.

Toxicity and consequences of exposure to selenium

Selenium accumulation is associated with agricultural irrigation, geochemical processes, mining, and a variety of other industrial sources and frequently results in significant effects on animal health (88). In California's San Joachim Valley, extensive agricultural irrigation has resulted in significant selenium contamination (40). Once an endpoint for this agricultural drainage, Kesterson National Wildlife Refuge serves as an example of the toxicological effects of selenium on wildlife, with a 64% rate of deformity and death of embryos and hatchlings of wild birds. Similar situations exist farther south in the Tulare Lake Bed area, the Salton Sea Area, and nine other regions in the western United States (89).

In other areas of the United States, power plant coal-fly ash receiving ponds create environments causing selenium toxicosis in wildlife (90, 13, 91). In addition to insect Se exposure through water and soil, many plants can sequester concentrations of sodium selenate, sodium selenite, selenocysteine and selenomethionine that can strongly influence insect development and survival. Some hyperaccumulator plant species have the ability to accumulate exceptionally high concentrations of Se on the order of 5000 μg/g dry wt, a level that even has negative effects on mammalian herbivores (92).

Several studies have demonstrated the toxicity of Se to insect herbivores fed Se amended diets (93, 94, 95, 96, 97), and Se-irrigated plants (98, 99, 100) at concentrations well within the range found in plants that are not considered hyperaccumulators. Trumble et al. (96) found LC_{50}s for the generalist herbivore *Spodoptera exigua* (Noctuidae) below 22 μg/g wet weight (<50 μg/g dry wt). At this level of toxicity all of the forms of Se tested were more potent against *S. exigua* than many compounds that are believed to have evolved for plant defence against insect herbivores (101, 102).

Relatively few reports are available on the potential ecological consequences of Se accumulation in host plants and herbivorous insects (98, 100). There is even less information on the effects of Se on the third trophic level (insect predators and parasitoids) which prey on other insects (103). Vickerman and Trumble (unpublished) conducted a study to determine potential effects on the life cycle of a predatory insect following consumption of Se-containing prey. While they did not find bioaccumulation from the prey to the predator, the effects from the selenium that did transfer to the predator were noteworthy. Feeding on herbivores that were given a diet containing Se significantly reduced predatory insect survival to the adult stage. In addition, predators that were fed Se-contaminated hosts weighed significantly less at the adult stage and had significantly decreased developmental rates. This mortality and delay in development time would decrease the intrinsic rate of population increase and expose the predators to additional stress from other environmental factors.

Selenium has been shown to accumulate preferentially in the malpighian tubules and the midgut of insects, but has also been shown to increase in the rest of the body after a saturation point is reached (94, 104, 105). This implies regulation through elimination (or sequestration if the selenium is stored permanently as in the 's' cells in isopods (3)), a process that is obviously overwhelmed when concentrations exceed tolerances. The resulting toxicity is attributed to the substitution of selenium for sulphur in amino acids, resulting in incorrect tertiary structure and malformed, non-functional proteins and enzymes (106, 107).

A significant problem concerning research on the ecological impact of selenium is

determining which form of selenium is causing the toxic effects and should be monitored (12). Fan et al. (13) showed that the organic forms of selenium, in particular selenomethionine, are a substantial component of the biomass selenium in the aquatic ecosystem. While this provides strong evidence, it is not conclusive regarding the form of selenium causing toxicity. Additional research is required to make more robust conclusions and predictions.

Future studies: The deficiency of information on chemical mixtures

Pollutants rarely occur in the natural environment in a pure form, and metals are no exception. Many of the recent studies reviewed in this chapter contain data for multiple metals (7, 51, 5, 68), but none of them examine the potential interactions between metals. These interactions might be additive, antagonistic, or synergistic and could significantly increase or decrease toxicity. Unfortunately, much of the previous research may not be able to address this problem. When several valent states exist for a given metal, there are often years of experiments that were performed before the technology developed to differentiate between valent states. These studies, while important steps in determining biological effects of metals, currently have limited value. Many authors continue to express levels as a function of the total metal concentration in order to make comparisons and draw conclusions with regards to past studies. This problem is even evident in the policies adopted by regulatory agencies. In a review of selenium research in aquatic systems, Hamilton (12) pointed out that, in the U.S.A., the national water criteria do not take into account potential interactions. Nonetheless, now that reasonable separation technology exists, we should seize every opportunity to further expand our understanding of the true intricacy that exists in the environment.

A second problem is the complexity of these experiments. As Cain et al. (9) indicated, the effects of simultaneous, multiple metal exposures that occur in nature are poorly understood. Many variables complicate research priorities; which metals, what concentrations of each metal, what test organism(s), and what exposure conditions should be tested. This is further convoluted by the fact that in some cases the interactions have been shown to be reversed by altering the exposure conditions (12). While emerging technology should make the detection and quantification of metal mixtures easier, making predictions regarding the effects of multiple metal exposures will remain a challenge until much more data have been collected.

Ecotoxicology and the need for life parameter studies of insects

In a review of population level effects of pesticides on arthropods, Stark and Banks (108) commented that 95% of the published studies that they reviewed used mortality and median lethal dose/concentration as a toxicological endpoint. They argued that measures of the rate of population growth result in more accurate assessments of the impact of toxicants because both lethal and sub-lethal effects of the toxicant are included. Similarly, Forbes and Calow (109) found that demographic toxicological studies of life table responses provided more accurate assessments of toxicity than lethal concentration estimates. A wide range of sublethal effects can be tested, including modification in life-

span, development rates, fertility, fecundity, sex ratio, and changes in such behaviors as feeding, searching, and oviposition.

This concept can also be applied to metals. While lethal effects are easy to measure, the sublethal effects are generally more time consuming and more difficult to document. For example, in our measure of the effects of hexavalent chromium on the phorid fly, a 96 or 120 hour acute toxicity test would have taken less than a week to complete, whereas our measure of development time to emergence occurred over four weeks. While the short-term acute test allows for a quick assessment and comparability between species or toxicants, short term tests are likely to underestimate of the true effect of the toxicant. Indeed, Bechman (110) found that some toxicants can affect populations enough to cause extinction at concentrations well below the traditional dose-response curve. Thus, the longer tests, while certainly less efficient, may detect much more subtle effects and have more applicability in the field. Ultimately, incorporation of both survivorship and fecundity will provide increased resolution and ecological relevance to studies examining biological effects of metal pollution.

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