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Differentiation in Copper and Nickel Accumulation in Adult Female and Juvenile *Porcellio spinicornis* from Contaminated and Uncontaminated Sites in Northeastern Ontario

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Environmental metal pollutants exert strong selective pressures on organisms, offering unique opportunities for studying the phenomenon of metal tolerance (physiological acclimation), or resistance (genetic adaptation), in the natural polpulations. The selective agent, selection strength, and the timing of the selection process are often well known and easy to quantify. In most instances, according to Klerks and Livington (1989), selective pressures may be so strong that they make the effects visible during very short time, e.g., within two to three generations. Furthermore, according to Macnair (1987), the evolution of resistance to pollutants may illustrate the processes involved in early stages of speciation .

The evolution of tolerance to metals is well documented in aquatic invertebrate species (McNeilly and Bradshaw 1968; Lavie and Nevo 1982, 1986; Nevo *et al.* 1984; Klerks and Weiss 1987; Klerks and Levington 1989; and Posthuma 1990). However, similar information on terrestrial animal species is restricted to the studied reviewed by Hopkin (1989). Brown (1976) described metal tolerance in terms of acute toxicity tests and sub-lethal growth-rate studies in the freshwater isopod *Asellus meridianus* from rivers Hayle and Gannel which have received drainage from a copper-mine for the last 200 years. The data on the evolution of resistance to a combination of Cd, Ni and Co in the deposit feeding annelid *Limnodrilus hoffmeisteri* have been reported by Klerks and Levington (1989).

In earlier studies, Alikhan and Storch (1990) demonstrated the relationship of tissue Cu and Ni concentrations in whole isopods to their levels in the environment, and showed that metal granules were stored inside the membrane-bound lysosomal vesicles of the S - cell of the hepatopancreas. The present report provides information on the differentiation in copper and nickel accumulations in adult female *Porcellio spinicornis* Say, 1818 (Porcellionidae, Isopoda, Crustacea) obtained from both contaminated and uncontaminated sites in Northeastern Ontario, and their progeny.

MATERIALS AND METHODS

Adult seventh growth-stage *Porcellio spinicornis* were collected during July and August 1989 from wooded areas near the town of Copper Cliff (46°26' N 81°07' W), Ontario, Canada, and the Cup and Saucer Mountain on the Manitoulin Island near the town of Little Current (45°58' N 81°55' W), Ontario, Canada. Manitoulin Island is an uncontaminated site, whereas Copper Cliff sites are approximately 2 km downwind of the primary smelting works of the International

Nickel Company of Canada, and are heavily contaminated with cadmium, copper, nickel, manganese and zinc (Alikhan *et al.* 1990). Growth-stage of the isopod was determined by the criterion of Alikhan (1972).

Twenty-five ovigerous females from each population were transferred to individual plastic vials (4.5 cm diameter x 7.5 cm depth), lined at the bottom with wet gypsum, and kept at 23^{o}C, 86 - 96 per cent relative humidity, and a light (3.2 klx) - dark cycle of 14h:10h (light 5:30-19:30). These females and their progenies were provided with fresh pieces of apples (7.2 ± 0.5 μg Cu and 0.6 ± 0.09 Ni g^{-1} dry weight) every week. Apple residues were removed, oven - dried at 60^{o}C (± 5^{o}C), and weighed to the nearest 0.01 mg to determine the dry weight of the weekly food consumption for each isopod. To avoid fungal growth, fecal pellets were removed daily. The progeny of these twenty-five females was observed for 300 days, during which time the F_2 females had matured and release their youngs. Ten adult females, twenty-five 24-hr old mancas and 25 six - wk old juveniles from each generation up to three generations from each population were killed and kept at -5^{o}C until analyzed for metals.

Apple pieces, leaf litter from each collection site, whole adult females and their young (24 hr old mancas and 6 wk old juveniles) were oven dried at 60^{o}C for 12 hr. After cooling to room temperature, samples were weighed, digested in boiling concentrated aqua regia (3mL Merck's "suprapur" 65% HNO_3 : 1 mL BDH analytical grade concentrated HCl), diluted to 20 mL with 1*M* HNO_3, and analyzed for Cu and Ni with a Perkin-Elmer atomic absorption spectrophotometer by the flame method. The detection limits of the atomic absorption spectrophotometer used for Cu and Ni amounted to 0.077 and 0.15 μg mL^{-1}, respectively.

A two-way ANOVA evaluated effects of dietary metal concentrationss and the collection-sites. Within dietary metal concentrations and tissue concentrations comparisons were made using one-way ANOVA with Duncan's New Multiple Range test. All data were checked for normality (Kolmogorov - Smirnoff test) and homogeneity of variance (Bartlett - Box F - test), and they were log transformed, whenever necessary.

RESULTS AND DISCUSSION

Leaf litter from the Copper Cliff sites contained approximately 12 times more Cu (180 ± 8.8 μg g^{-1} vs 15.0 ± 0.8 μg g^{-1}) and 28 times more Ni (168.0 ± 8.4 μg g^{-1} vs 6.0 ± 03 μg g^{-1}) than did that procured from Manitoulin Island. This was reflected in the body tissue metal concentrations of the isopods obtained from the two sites. Thus, the females from Copper Cliff sites contained twice as much Cu (1137 ± 56.9 μg g^{-1} vs 685 ± 34.2 μg g^{-1}) and approximately 1.5 times more nickel (302.0 ± 15.1 μg g^{-1} vs 201 ± 10.1 μg g^{-1}) than did animals collected from Manitoulin Island. The metal concentration factor (calculated by dividing the animal tissue metal concentrations by metal concentrations in the leaf litter) for Cu in isopods obtained from the Manitoulin Island and the Copper Cliff sites amounted to 45.7 and 6.3, respectively, whereas those for Ni were 33.5 and 1.8, respectively.

Whole mancas analyzed within 24 hr of their release from the brood pouch, irrespective of the collection site their mothers were derived from, had approximately the same concentration of Cu or Ni ($P > 0.05$) (Table 1). However, 6-wk old nymphs released by females collected from Copper Cliff site had more than twice the tissue concentration of Cu and approximately 1.5 times the concentration of Ni than did juveniles reared from F_1 or F_2 females obtained from Manitoulin Island site (Fig. 1). Concentration factors for Cu and Ni in 24 hr

TABLE - 1. Copper and nickel contents of young *Porcellio spinicornis* within 24 hours of release from the brood pouch (µg g-1 dry weight, means of 25 individuals in each case).

Collection site	Progeny	Copper	Nickel
Manitoulin Island	F1	69.0 ± 3.45^a	7.8 ± 0.53^b
	F2	64.7 ± 3.10^a	6.6 ± 0.71^b
	F3	67.4 ± 2.89^a	7.2 ± 0.62^b
Copper Cliff	F1	68.2 ± 3.07^a	7.6 ± 0.43^b
	F2	66.0 ± 3.10^a	6.7 ± 0.63^b
	F3	65.6 ± 3.28^a	6.9 ± 0.56^b

Means within each column followed by the same letter are not significantly different at the 5 per cent level.

old mancas released by females collected from Manitoulin Island ranged from 4.3 to 4.6 and 1.1 to 1.3, respectively, whereas concentration factors for mancas released by females from the Copper Cliff site ranged from 0.36 to 0.37 and 0.03 to 0.04, respectively (Table 1). Concentration factors for Cu and Ni for the 6 - wk old nymphs from Manitoulin Island site ranged from 17.0 to 19.6 and 4.01 to 4.81, respectively, whereas those from Copper Cliff site ranged from 4.28 to 4.45 and 0.59 to 0.65, respectively.

This study demonstrates that both Cu and Ni are taken up by the terrestrial isopods from their environment, and that the metal which is not immediately utilized or excreted by the animal is stored in the various body tissues. In general, isopods store rather than excrete metals to save energy (for complete discussion of the concept, see Simkiss 1977). The concentration factors for Cu and Ni, in the present study, depending upon their background levels in the environment, ranged from 0.36 to 19.6 and from 0.03 to 4.81, respectively. These concentration factors compare well with those reported for the two metals for other isopod species from a wide range of contaminated and uncontaminated sites (Hopkin and Martin 1982, 1984). In fact, the positive correlation of metal levels in the leaf litter with metal concentrations in whole isopods has led to the suggestion that terrestrial isopods are ideal and reliable "biological monitors" of environmental contamination by metals (Hopkin et al. 1986; Dallinger et al. 1992). However, the results of the present study appear to challenge this suggestion.

The data in the present study show that isopod populations originating from contaminated sites accumulate metals in significantly larger amounts than those from uncontaminated sites (a positive correlations with levels in leaf litter). Furthermore, the study also shows that these differences in metal accumulation by the two populations are maintained at least up to three generations even when

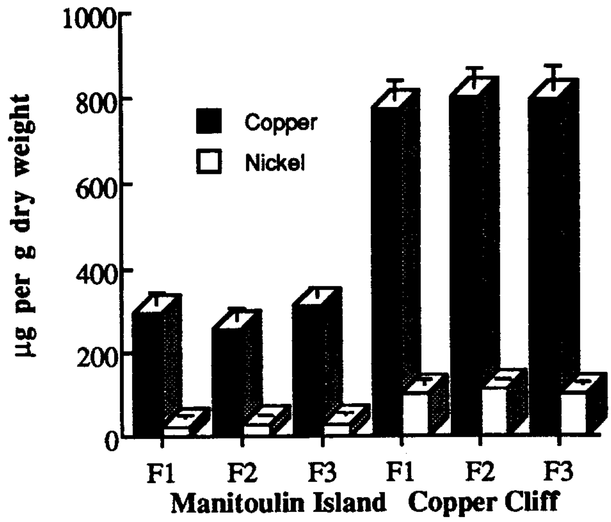

Figure 1. Copper and nickel contents in juvenile *Porcellio spinicornis* after feeding for six weeks on apple. Treatments within each site were not significantly different from each other at the 5 per cent level.

the animals are fed on the same diet. Thus, there is a possibility that "genetic factors" in isopod populations may control the extent to which Cu and Ni are accumulated in the tissue. However, more experimental evidence will be required to support this contention.

According to Klerks and Weis (1987), there may be two reasons why organisms are resistant to a pollutant : (i) individual organisms may have acquired a degree of tolerance by physiological acclimation during exposure to sublethal concentrations at some prior period of their lives. The presence of physiological tolerance to sublethal concentrations in parents does not, however, confer physiological tolerance to lethal concentrations upon the offspring, which must also be pre-exposed to acquire it; (ii) populations may have evolved genetically-based resistance through the action of natural selection on genetically-based individual variation in resistance. Both phenomena have been extensively studied in the development of resistance in insect pests to insecticides. It is possible that similar factors may work to impart genetically-based resistance to metals in terrestrial isopods. However, more detailed data from strictly controlled experiments will be needed to support this contention.

The increased resistance to Cu and Ni exhibited in the present study by isopod females from the contaminated site, as well as by their offspring, appears somewhat different from the phenomenon of co-resistance shown by organisms exhibiting tolerance to another metal of the same chemical group. Such resistance has been demonstrated in Zn-tolerant *Agrotis tenuis* for Zn and Ni (Gregory and Bradshaw 1965), both of which are borderline metals between class A and class B elements (Niebeor and Richardson 1980). Co-resistance to metals which utilize different processes (or carrier molecules) to gain entry into the cell, are rare. However, co-resistance to Cu (a class B, sulphur- or nitrogen-seeking element, Nieboer and Richardson 1980) and Pb (a borderline metal between class A and class B elements, Nieboer and Richardson 1980) has been known to occur in the freshwater isopod, *Asellus meridianus* (Brown 1976). The mechanism of resistance against Cu and Ni (a borderline element, Nieboer and Richardson 1980) in *Porcellio spinicornis* may operate in an identical manner; adaptation to one metal affecting the uptake of the other. Copper, a regulated metal and an integral part of the isopod respiratory protein, hemocyanin, according to Alikhan and Storch (1990), is stored in lysosomal vesicles of the 'S' cells of the hepatopancreas. Nickel, on the other hand, is not a true intracellular uptake but probably represents metal bound at the cell surface (Alikhan and Storch 1990). Thus, it appears that crustacean cells deal with these two metals through different metabolic pathways. Bardeggia and Alikhan (1991) have recently demonstrated that Cu accumulation by whole crayfish is retarded by the presence of approximately 100 μg Ni g^{-1} wet weight the diet, whereas increasing Ni to 500 - 600 μg g^{-1} wet weight enhances it. This implies that Cu and Ni, depending upon their levels in the environment, may act both as synergists and antagonists towards each other. Effects of Ni on the stretch receptor action potential in the crayfish have shown that Ni^{2+} and Ca^{2+} (a class A, oxygen-seeking, element) may compete for the same membrane site (Washizu 1965). One could, therefore, argue that the same type of competition may exist between Cu and Ni. However, such simple arguments cannot explain all incidences of synergism and antagonism between Cu and Ni, and a more detailed study is needed to offer reasonable explanation for the phenomenon.

Fraser *et al.* (1978) presented data on lead tolerance in the isopod, *Asellus aquaticus*, from a site with high levels of Pb, and have compared it with conspecifics from two control sites. Their results, however, did not contain convincing evidence to attribute their observations to either acclimation (= tolerance) or adaptation (= resistance). Nevertheless, they claimed that the differential survival recorded between large and small individuals might be explained in terms of genetic adaptations, the larger and older individuals being more resistant due to selection taking place prior to that stage. Such variations in tolerance, it seems, need not have a genetic component.

Lavie and Nevo (1986) have demonstrated that exposure to Hg, Zn, Cd, Pb and Cu may lead to a differential survival of certain genotypes in the shrimp *Palaemon elegans*. Similar results were reported by Nevo *et al.* (1984) who predicted that mercury-resistant allozymes would occur at higher frequencies at a mercury-polluted site. They found a relatively higher (although not quite statistically significant; $p = 0.089$) allozyme occurrence frequency at the polluted site of the genotype shown in the laboratory to be superior at low and intermediate Hg levels. However, they were unable to demonstrate differences for a genotype shown in the laboratory to be favored at the higher Hg levels. It is possible that a natural selection of individual for increased resistance to Hg may have occurred at the contaminated site. More detailed work to obtain experimental evidence on the presence of genetic resistance in isopods to metals in the environment is in progress.

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