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Developing Standards for Environmental Toxicants: The Need to Consider Abiotic Environmental Factors and Microbe-Mediated Ecologic Processes

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This article suggests and discusses two novel aspects for the formulation of standards for environmental toxicants. First, uniform national standards for each pollutant will be underprotective for some ecosystems and overprotective for others, inasmuch as the toxicity of a pollutant to the indigenous biota is dependent on the physicochemical properties of the recipient environment. As the number of chemicals that need regulation is immense and as microbes appear to respond similarly to pollutant-abiotic factor interactions as do plants and animals, it is suggested that microbial assays be used initially to identify those abiotic factors that most influence the toxicity of specific pollutants. Thereafter, additional studies using plants and animals can focus on these pollutant-abiotic factor interactions, and more meaningful standards can then be formulated more rapidly and inexpensively. Second, it is suggested that the response to pollutants of microbe-mediated ecologic processes be used to quantitate the sensitivity of different ecosystems to various toxicants. Such a quantification, expressed in terms of an "ecological dose 50%" (EcD_{50}), could be easily incorporated into the methodologies currently used to set water quality criteria and would also be applicable to setting criteria for terrestrial ecosystems.

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Introduction

Through a variety of Federal statutes, including the Clean Air Act (CAA) of 1970, the Clean Water Act (CWA) as amended in 1977, the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) of 1972, the Federal Water Pollution Control Act (FWPCA) of 1972, the Resource Conservation and Recovery Act (RCRA) of 1976, and the Toxic Substances Control Act (TSCA) of 1976, the United States Environmental Protection Agency (EPA) is charged with protecting the health and welfare of human beings and of the environment from harmful exposures to toxic agents (*1*). For example, as required by the CAA, EPA set national primary standards to protect human health against toxic levels of atmospheric particulate matter, sulfur dioxide, nitrogen oxides, carbon monoxide, hydrocarbons, photochemical oxidants and lead and established national secondary standards for only particulate matter and sulfur dioxide (*2-5*). In 1979, EPA set new criteria for pollutants occurring in aquatic ecosystems. The Water Quality Criteria for 65 categories of chemicals considered toxic under the 1977 Amendments to the CWA were set at levels considered safe for human health and for various components of the aquatic biota (*6-9*). These criteria were later defined in terms of 129 specific priority chemicals that are to receive the maximum possible control in the discharge of effluents (*10*). As there is no "Clean Soil Act", EPA has not formulated criteria or standards for toxicants occurring in terrestrial environments. However, the level of toxicants in soils is indirectly monitored by the Federal Food, Drug, and Cosmetic Act (FFDCA), which requires EPA and the Food and Drug Administration (FDA) to set action levels and tolerances for permissible levels of toxicants in foods. In this manner, some pollutants entering the food chain from contaminated soils and then consumed by human beings are regulated (*11*). Furthermore, TSCA requires the preproduction testing of any new chemical or any existing chemical with new uses which "may present an unreasonable risk to health or the environment," including both aquatic and terrestrial ecosystems.

This article discusses two aspects involved in developing standards for toxicants as related to basic environmental safety but not directly to protecting

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human health. First, the toxicity of a pollutant to the indigenous biota is dependent, in part, on the physicochemical properties of the recipient environment. Because of the large number of chemicals that require testing, it is suggested that microbial assays be utilized as the initial screening system to identify those abiotic factors that influence most the toxicity of different chemical pollutants. Once these interactions have been identified, further testing should be with representative species of the macrobiota. Second, microorganisms in natural habitats, as well as many of the basic ecologic processes that are under the control of microbial activities and which are needed to maintain the quality of the biosphere, are sensitive to pollutants. However, when formulating standards for the toxicants mandated by the CAA and criteria for toxicants identified by the CWA, EPA did not consider the potential adverse effects of these toxicants on the various microbe-mediated ecologic processes (e.g., biogeochemical cycles, litter decomposition). Consequently, it is suggested that the adverse effects of toxicants on microbe-mediated ecologic processes be incorporated into the methodologies currently used to set environmental standards. The development of a new formulation, termed the EcD_{50} (i.e., the ecological dose 50%, which is the concentration of a toxicant that inhibits a microbe-mediated ecologic process by 50%), would greatly facilitate the incorporation of data on the adverse effects of toxicants to ecologic processes into the methodologies involved in regulatory legislation.

Physicochemical Factors: Modifiers of Pollutant Toxicity

High Risk Environments

Regulatory agencies that establish permissible levels for toxicants in foods, the workplace, and the environment have recognized the existence of hypersensitive subgroups within the general human population. These hypersensitive individuals are termed "high risk groups," and their sensitivity is determined, depending on the specific toxicant, by such biotic factors as nutritional status, genetic constitution, developmental stage, and overall health. For example, when setting action levels and tolerances for lead (Pb) in foods and milk, FDA recognized the hypersensitivity of infants and toddlers (*11*), and EPA, in its health assessment for cadmium (Cd), stated that "due to increased absorption of Cd being associated with certain nutritional deficiencies, e.g., insufficient levels of dietary iron (Fe), zinc (Zn), or calcium (Ca), older members of the population are likely to be at even greater risk" than the general population (*12*). The National Institute for Occupational Safety and Health (NIOSH), in its review of the scientific literature on occupational exposure to DDT that was prepared for the Occupational Safety and Health Administration (OSHA), noted that "female workers exposed to DDT and other pesticides are reported to have suffered a significantly higher frequency of miscarriages and prepartum disorders than less exposed controls" (*13*).

The toxicity of an environmental contaminant to the biota is influenced, in part, by the physicochemical properties (Table 1) of the recipient environment. The toxicity of a pollutant may be reduced by the specific abiotic properties of one ecosystem, whereas in another ecosystem with different physicochemical characteristics, the toxicity of an equivalent dose of the same pollutant may be potentiated (*14-17*). The latter environments should be considered high risk environments. High risk groups and high risk environments are essentially similar concepts: a high risk group is a population for which the toxicity of a pollutant is magnified; a high risk environment is an ecosystem in which the toxicity of a contaminant to the indigenous biota is magnified. Consequently, just as regulatory agencies recognize high risk groups when establishing safe levels of contaminants in foods, the environment, and the workplace, similar consideration should be directed to identifying high risk environments (*18*).

Table 1. Physicochemical factors of an environment that can affect the toxicity of pollutants.

Factor
pH (acidity/alkalinity)
E_h (oxidation-reduction potential)
Aeration status (aerobic, microaerobic, anaerobic)
Buffering capacity
Inorganic anionic composition
Inorganic cationic composition
Water content
Clay mineralogy
Hydrous metal oxides
Organic matter
Cation exchange capacity
Anion exchange capacity
Temperature
Solar radiation
Hydrostatic pressure
Osmotic pressure

Most standards for toxicants are based on a series of assumptions, e.g., that the response of rodents to a toxicant can be extrapolated to setting a standard for that toxicant suitable to protect human beings; that the response of a few test species to a toxicant can be extrapolated to setting a standard that will protect the multiplicity of life in an entire ecosystem against that toxicant. If the assumptions are either incorrect or incomplete, such as result of the failure to recognize the existence of high risk

environments, then the regulations based on these assumptions will be inappropriate to provide proper protection (*19*). A standard for an environmental toxicant that is based on only one set of abiotic environmental variables may be overprotective or underprotective for ecosystems with differing physicochemical properties (*1, 15-17*).

To illustrate how physicochemical environmental factors influence pollutant toxicity, some heavy metal pollutants will be used as examples. Physicochemical factors may influence the toxicities of heavy metals by affecting their (a) chemical speciation form, (b) chemical/physical mobility and (c) bioavailability. For example, in marine environments, Cd occurs primarily as a mixture of $CdCl^+/CdCl_2/CdCl_3^-$, whereas in acidic or neutral fresh waters it occurs as Cd^{2+} (*20*). Similarly, in marine ecosystems, mercury occurs primarily as a mixture of $HgCl_3^-/HgCl_4^{2-}$, whereas in fresh waters, depending on the pH, it occurs as Hg^{2+}, $HgOH^+$, or $Hg(OH)_2$. The different inorganic speciation forms of these metals exert differing toxicities. Fungi tolerated Cd (*21*) and bacteria and bacteriophages tolerated Hg (*22*) better in marine environments or in synthetic media with a level of chlorinity comparable to that occurring in oceans than in fresh waters or in synthetic media with a limited chloride content, thus indicating the lesser toxicities of the metal chloride species.

Several abiotic factors limit the chemical/physical mobility of heavy metals. Heavy metals that are immobilized, e.g., by sorption to clay minerals and other particulates or by precipitation as phosphate, carbonate, or sulfide salts, are less readily available for uptake by the biota. For example, incorporation of the clay minerals, montmorillonite and kaolinite, into synthetic media (*23*) or soil (*24*) decreased the toxicity of Cd to bacteria and fungi. The incorporation of montmorillonite, attapulgite or kaolinite, of particulate organic matter, or of carbonate or phosphate into a synthetic medium decreased the toxicity of Pb to fungi (*25*). Nickel and Cd were less toxic to fungi in hard water than in soft water, probably as a result of the higher levels of carbonate and magnesium in the hard water (*26, 27*).

Inorganic cations present in various environments may influence the bioavailability and uptake of heavy metals to the biota. Competition for sites on the cell surface between cations normally present in a specific habitat and the cationic forms of the heavy metals may reduce the toxicity of the heavy metals. For example, the toxicity of Ni to marine fungi was reduced in the presence of seawater. At the pH and chlorinity of seawater, Ni occurs as Ni^{2+}, and the reduction in Ni toxicity was correlated with the Mg content of seawater, indicating that competition between Ni and Mg, which have similar ionic radii, for common sites on the cell surface reduced the uptake and, hence, toxicity of Ni (*28*). The other abiotic factors listed in Table 1 also differentially affect the toxicity of heavy metals (*15, 16*).

An environment that may be high risk for one pollutant may be of low risk for a different pollutant. For example, the toxicity of Cd (*24, 29*) and Zn (*30*) to microorganisms was decreased in acidic systems, whereas that of Pb (*25*) and Ni (*31, 32*) was increased. There was no consistent relation between the toxicity of Mn and the pH of the medium (*33*), and the toxicity of Hg was pH-independent (*30*).

The Water Quality Criteria that were suggested by EPA indicate that regulatory agencies have begun, although only to a limited extent, to recognize that the toxicity of pollutants is dependent on the abiotic characteristics of the recipient environment. In formulating these criteria, EPA noted that "the toxicity of certain compounds may be less in some waters because of differences in acidity, temperature, water hardness, and other factors. Conversely, some natural water characteristics may increase the impact of certain pollutants." Consequently, separate criteria were set for fresh and marine ecosystems. Furthermore, as the toxicity of heavy metals appears to be directly related to the degree of hardness in fresh waters, the criteria for Cd, Pb, Ni, Zn, Cu, Cr, and Be were formulated to reflect this "sliding scale", i.e., as the hardness increases, the level of the metals that can be tolerated by the biota also increases (*6-8*). For example, for hardness levels of 50, 100, and 200 mg/L as $CaCO_3$, the criteria for Cd are 0.012, 0.025, and 0.051 μg/L, respectively (*9*)

Although other environmental factors influence the toxicity of heavy metals (as well as of other pollutants), EPA considered only the level of hardness in fresh waters. For the metals that were evaluated by EPA, the allowable levels were higher in marine than in fresh waters and in hard than in soft waters, indicating that the highest risk, or most fragile, ecosystems for heavy metal pollutants would be soft fresh waters. The focus by EPA on only hardness reflects the lack of sufficient data to establish relationships between other abiotic factors and pollutant toxicity. "Although EPA recognizes that other water characteristics such as pH, temperature, or degree of salinity (as in estuaries) may affect the toxicity of some pollutants, the data base at this time is not detailed enough for further specificity". EPA further stated that these criteria will not be "cast in concrete" but will be updated in future years when additional information becomes available (*6*).

There is, therefore, a critical need for additional

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information on the influence of physicochemical factors on pollutant toxicity. The continued lack of such data will result in criteria that are inappropriate (e.g., they will be either under- or overprotective). Although the number of abiotic factors (Table 1) and their interactions that can modify pollutant toxicity may appear to be too complex to incorporate successfully into standards that can easily be formulated and interpreted, not all these factors are of equal importance in each ecosystem, and not all of the abiotic factors influence significantly the toxicity of each pollutant. Most ecosystems possess distinct abiotic factors that dominate and serve to characterize those environments. For example, alkaline pH and high inorganic ion content are the dominant characteristics of surface marine waters, and high cation exchange capacity, high organic matter content and acidic pH are the dominant characteristics of peat soils. Consequently, only the modifying influence of the dominant abiotic factors of specific environments on pollutant toxicity probably need be considered in the regulatory decision-making process. Furthermore, for most chemicals, perhaps only two or three abiotic factors will significantly modify their toxicity. For example, pH and buffering capacity appear to be the abiotic factors that most influence the harmful effects of acid precipitation (*54*). Consequently, once the dominant abiotic factors that influence the toxicity of a specific pollutant and the relative importance of these factors in different ecosystems have been established, the environmental analyst need focus only on those abiotic factors. The establishment of a positive correlation between the dominant abiotic factors of ecosystems with those abiotic factors that most significantly modify the toxicity of a pollutant (as determined in laboratory screening) should aid in formulating criteria that would protect all ecosystems against that pollutant. For example, if the toxicity of a pollutant is reduced by high pH and salinity, then distinct criteria should be set for marine and fresh water ecosystems, with the latter being the high risk environment. Conversely, if the toxicity of a chemical is not affected by pH or salinity, one criterion for both fresh and marine water would perhaps provide suitable protection for both ecosystems.

Microbes as Assay Systems

Most research on chemical toxicants has focused on identifying the effects on human health of both acute and, to a lesser extent, chronic exposures and on identifying the molecular bases of the adverse responses. There has been only limited research to evaluate the interactions between pollutants and abiotic environmental factors and the resultant effects of these interactions on the general biota. It is the lack of such data that has hindered EPA in setting criteria that are reflective of the different types of ecosystems in the United States. The volume of chemicals that need such evaluations—e.g., 129 just for the Water Quality Criteria and an estimated 63,000 already in commerce plus approximately 1,000 new ones estimated annually (*35*), the limitations in laboratory facilities (especially if microcosms are used) and trained personnel, the expensive costs, and the need for "rapid" results has prompted our recommendation for using microbes as assay systems to identify those abiotic factors that most significantly influence the toxicity of specific chemicals.

Microbes can serve as adequate monitors to predict the response of the microbiota to a toxicant as influenced by abiotic factors. For example, a compilation of data of the responses to Cd by representatives of the aquatic macrobiota (Table 2), terrestrial macrobiota (Table 3) and microbiota (Table 4) indicates common biologic responses (as well as similar contradictions in data) among these three distinct groups to Cd toxicity as influenced by abiotic factors. Microbial assays should be used initially to identify which environmental variables, singly or in various combinations, most directly affect the toxicity of a specific chemical. Once these variables have been clearly identified for specific chemicals, further studies with representative species of the macrobiota can be performed, and criteria or standards can be formulated on the basis of their results.

The use of microbial assays to predict the toxicity of chemicals to the macrobiota, including human beings, is not novel. Chronic effects, such as genetic diseases, birth defects and cancer, appear years or even decades after the initial exposure to the toxicant, and long-term studies using animals must be conducted to detect these latent responses. Such studies are expensive: for example, a single test to determine the potential carcinogenicity of a chemical may require as long as three years at a cost of $250,000 or more. Furthermore, the "world laboratory capacity" for such chronic studies is estimated at 500 chemicals/year, which is not sufficient to keep pace with the 700 to 1000 chemicals introduced annually into commerce (*98*). In response to these difficulties, short-term tests [the best known being the Ames' test (*98*)] with bacteria, yeasts, filamentous fungi, plants, insects, and isolated mammalian cells have been developed and are used as rapid and relatively inexpensive predictors of a chemical's potential to cause adverse chronic effects (*99*).

There is, therefore, a need for microbial assays, not only to screen chemicals for their potential chronic effects on human beings, but also to identify which abiotic factors most influence their toxicity in

Table 2. Physicochemical factors affecting the toxicity of cadmium to the aquatic biota.

Environmental factor	Comments	Reference
Temperature	The estuarine fish, *Fundulus heteroclitus* was more sensitive to Cd at 20°C than at 5°C	(36)
	Fingerlings of the freshwater perch, *Perca fluviatilis*, accumulated more Cd at 15°C than at 5°C	(37)
	The estuarine crab, *Paragrapsus gaimardii*, was more sensitive to Cd at 19°C than at 5°C	(38)
	The American oyster, *Crassostrea virginica*, accumulated more Cd at 20°C than at 5°C	(39)
Salinity	Increasing the salinity decreased the toxicity of Cd to the grass shrimp, *Palaemonetes pugio*	(40)
	The toxicity of Cd to the blue crab, *Callinectes sapidus*, decreased with increasing salinity	(41)
	The toxicity of Cd to marine and estuarine crustaceans increased as the salinity was decreased	(42)
	The marine mussel, *Mytilus edulis*, accumulated more Cd at 11 than at 30 ‰ salinity	(43)
	Fundulus heteroclitus was more sensitive to Cd at 5 ‰ salinity than at 15 to 35 ‰ salinity	
Water hardness	The rainbow trout, *Salmo gairdneri*, tolerated more Cd as the water hardness was increased	(44), (45)
	The fathead minnow, *Pimephales promelas*, tolerated Cd better in hard than in soft water	(46)
	The toxicity of Cd to the brook trout, *Salvelinus fontinalis*, decreased as the water hardness was increased	(47)
	The freshwater snail, *Ampullaria paludosa*, the catfish, *Corydoras punctatus*, and the guppy, *Lebistes resticulatus*, accumulated more Cd in soft than in hard water	(48)
	Increasing the water hardness decreased the toxicity of Cd to eggs of the teleost, *Oryzias latipes*	(49)
Inorganic cations	Simultaneous exposures to Pb, Zn, and Cu reduced the uptake of Cd by the freshwater plant, *Elodea nuttallii*	(50)
	Ca decreased the toxicity of Cd to the marine amphipod, *Marinogammarus obtusatus*	(51)
	Zn reduced the toxicity of Cd to *Pimephales promelas*	(52)
	Ca reduced the toxicity and uptake of the Cd by the freshwater shrimp, *Gammarus pulex*	(53)
Inorganic anions	Pyrophosphate reduced the uptake of Cd by *Daphnia magna*	(54)
Organic matter	Colloidal organic particulates decreased the toxicity of Cd to the freshwater crustacean, *Simocephalus serrulatus*	(55)
	Humic acid reduced the uptake of Cd by *Crassostrea virginica* and by *Daphnia magna*	(39, 54)
Synthetic chelators	NTA reduced the toxicity of Cd to *Palaemonetes pugio*	(40)
	EDTA and NTA reduced the uptake of Cd by *Daphnia magna* and by *Crassostrea virginica*	(39, 54)
	EDTA, NTA, and DTPA reduced the uptake of Cd by the carp, *Cyprinus carpio*	(56)
	EDTA reduced the uptake of Cd by the marine barnacle, *Semibalanus balanoides*	(57)

natural environments. Just as the results of microbial assays are used to make more informed decisions as to which chemicals should be examined further in the limited number of laboratories equipped for performing chronic toxicity studies with whole animals, microbial assays should be used to determine which abiotic factor-pollutant interactions should be studied further with representative species of the macrobiota in either simplified artificial systems or in complex microcosms.

Protecting the Environment *In Toto*

Microbe-Mediated Ecologic Processes

Attention by environmental policy-makers responsible for regulating toxicants has focused, and rightfully so, on human health, as evidenced by the numerous federal statutes concerned with limiting the exposure of human beings to harmful chemicals (e.g., CAA, CWA, FIFRA, FWPCA, FFDCA, RCRA, TSCA). However, the continued health and welfare of human beings is dependent on maintaining the quality of the biosphere, as acknowledged in TSCA, which requires the preproduction testing of new chemicals and the testing of existing chemicals with new uses for their potential hazards to the environment. As stated in TSCA, "It is the policy of the U.S. that adequate data should be developed with respect to the effect of chemical substances and mixtures on health and the environment." Regulatory agencies and environmental policy analysts appear to have narrowly defined "effect on the environment" as direct effects on the biotic components of the biosphere and have not considered the effects of pollutants on ecologic processes mediated by the biotic component and which are necessary to maintain the present state of the environment. For example, EPA has stated that the Water Quality Criteria were intended "to reflect the latest scientific knowledge on the identifiable effects of pollutants on public health and welfare, aquatic life, and

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Table 3. Physicochemical factors affecting the toxicity of cadmium to terrestrial plants.

Environmental factor	Comments	Reference
pH	Uptake of Cd by oats and lettuce increased as the pH was decreased	(58)
	Uptake of Cd by corn was independent of soil pH	(59)
	Increasing the soil pH from 5.5 to 7.5 reduced uptake of Cd by rice	(60)
	Chard and tomato accumulated more Cd when grown in acidic (pH 5.0 to 5.7) than in alkaline (pH 7.5 to 7.8) soils	(61)
	Increasing the soil pH from 4.5 to 6.4 reduced the uptake of Cd by ryegrass and oat	(62)
Temperature	Uptake of Cd by soybeans increased as the soil temperature was increased	(63)
Salinity	Increasing the salinity from 0 to 10 ‰, decreased, but from 10 to 30 ‰, increased, the toxicity of Cd to germination of seeds of *Spartina alterniflora*	(64)
Cation exchange capacity	Uptake of Cd by oat was lower in soils with high than with low cation exchange capacities	(65)
Water content	Increasing the water content of the soil increased the uptake of Cd by barley	(66)
	No synergistic interaction was noted between a drought stress and Cd for growth of *Andropogon scoparius*, *Monarda fistulosa*, and *Rudbeckia hirta*	(67)
Nitrogen content	Uptake of Cd by fescue, grown in soil, was enhanced by nitrogen amendments	(68)
	Uptake of Cd by bush bean, grown in a nutrient solution, was decreased by nitrogen amendments	(69)
Inorganic cations	Uptake of Cd by oat and lettuce, grown in a nutrient solution, was decreased by the addition of Ca, K, or Al	(58)
	Synergism was noted between Cd and Pb in reducing root growth, woody stem diameter growth, and foliage growth of American sycamore	(70)
	Synergism was noted between Cd and Pb in reducing vegetative growth of corn shoots	(71)
	Al reduced the uptake of Cd by *Hokus lanatus*	(72)
	Ni or Pb added to soil increased the uptake of Cd by ryegrass	(62)
Inorganic anions	Uptake of Cd by oat, grown in soil, was decreased by the addition of phosphate	(73)
	Phosphate amendments decreased the uptake of Cd by corn seedlings	(74)

Table 4. Physicochemical factors affecting the toxicity of cadmium to the microbiota.

Environmental factor	Comments	Reference
pH	Increasing the pH from 5 to 9 progressively increased the toxicity of Cd to *Aspergillus niger*, from pH 7 to 9 increased the toxicity of Cd to *Bacillus cereus*, *Alcaligenes faecalis*, and *Trichoderma viride*, and from pH 8 to 9 increased the toxicity of Cd to *Agrobacterium tumefaciens*, *Nocardia paraffinae*, and *Rhizopus stolonifer*; pH did not affect the toxicity of Cd to *Streptomyces olivaceus*	(29)
	Increasing the soil pH from 5.1 to 7.2 increased the toxicity of Cd to mycelial growth of *Aspergillus niger* but not of *Aspergillus fischeri*	(24)
	Increasing the pH from 6 to 8 increased the toxicity of Cd to *Micrococcus luteus*, *Staphylococcus aureus*, *Clostridium perfringens*, *Escherichia coli*, and *Pseudomonas aeruginosa*; pH did not affect the toxicity of Cd to *Bacillus subtilis*	(75)
	Increasing the pH from 6.5 to 8.3 increased the toxicity of Cd to *Chlorella pyrenoidosa*	(76)
	Cd toxicity to *Chlorella pyrenoidosa* decreased as the pH was increased from 7 to 8	(77)
	Uptake of Cd by the diatom, *Navicula pyrenoidosa*, and the green alga, *Chlorella pyrenoidosa*, increased as the pH was increased from 6 to 8	(78)
	Increasing the pH from 6 to 9 decreased the toxicity of Cd to the cyanobacterium, *Nostoc calcicola*	(79)
	The pH-Cd toxicity interaction towards mycelial growth of the fungi, *Achyla* sp. and *Saprolegnia* sp., was dependent on the composition of the growth medium	(30)
Temperature	*Chlorella pyrenoidosa* accumulated Cd faster at 25°C than at 4°C	(76)
	Chlorella pyrenoidosa accumulated Cd faster at 15°C than at 5°C	(80)
Water hardness	The alga, *Nitella flexilis*, accumulated more Cd in soft than in hard water	(48)
	Rhizopus stolonifer, *Scopulariopsis brevicaulis*, *Penicillium vermiculatum*, *Trichoderma viride*, *Beauveria* sp., and *Aspergillus niger* tolerated Cd better in hard than in soft water	(26)

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Table 4 (Continued)

Environmental factor	Comments	Reference
Salinity	Increasing the salinity above 45 °/oo reduced the toxicity of Cd to an unidentified marine bacterium	(81)
	The toxicity of Cd to *Rhizopus stolonifer*, *Trichoderma viride*, *Aspergillus niger*, and *Arthrobotrys conoides* was reduced in medium amended with seawater at 20% or greater	(21)
Synthetic chelators	EDTA decreased the toxicity of Cd to the marine diatom, *Ditylum brightwellii*	(82)
	NTA reduced the toxicity of Cd to photosynthesis of a natural freshwater phytoplankton community	(83)
	EDTA reduced the toxicity of Cd to *Klebsiella pneumoniae*	(84)
	EDTA reduced the toxicity of Cd to *Nostoc calcicola*	(79)
Organic matter	Pyruvate, gluconate, citrate, and aspartate reduced the toxicity of Cd to *Klebsiella aerogenes*	(84)
	Increasing the concentration of peptone decreased the toxicity of Cd to an unidentified marine bacterium	(81)
	Citrate increased the toxicity of Cd to *Pseudomonas* sp. but not to *Escherichia coli*	(85)
	Glutamine and cysteine decreased, but citrate increased, the toxicity of Cd to *Nostoc calcicola*	(79)
	Humus reduced the toxicity of Cd to *Selanastrum capricornutum*	(86)
Clay minerals	Montmorillonite and, to a lesser extent, kaolinite decreased the toxicity of Cd to *Bacillus megaterium*, *Agrobacterium tumefaciens*, *Nocardia corallina*, *Fomes annosus*, *Pholiota marginata*, *Botrytis cinerea*, *Aspergillus niger*, *Phycomyces blakesleeanus*, *Trichoderma viride*, *Chaetomium* sp., *Thielaviopsis paradoxa*, *Scopulariopsis brevicaulis*, and *Schizophyllum* sp. in synthetic medium	(23)
	Montmorillonite and, to a lesser extent, kaolinite protected *Penicillium vermiculatum*, *Aspergillus asperum*, *Aspergillus niger*, *Aspergillus fischeri*, and *Trichoderma viride* against Cd toxicity in soil	(24)
Cation exchange capacity	Cd was less toxic to *Penicillium vermiculatum*, *Penicillium asperum*, *Aspergillus niger*, *Aspergillus fischeri*, and *Cunninghamella echinulata* when grown in an alkaline soil with a high cation exchange capacity (i.e., 16 meq/100 g) than in an acid soil with a low cation exchange capacity (i.e., 8.2 meq/100 g)	(20)
Inorganic cations	Mg reduced the toxicity of Cd to growth of *Escherichia coli*	(87)
	Se reduced the toxicity of Cd to growth of *Haematococcus capensis*	(88)
	The toxicity of Cd to growth of *Aspergillus niger* was decreased by Ca and Mg	(89)
	Zn decreased the toxicity of Cd to growth of *Euglena gracilis*	(90, 91)
	Mn inhibited the uptake of Cd by *Chlorella pyrenoidosa*	(80)
	Cd and Pb interacted synergistically towards inhibiting growth of a brackish water phytoplankton community	(92)
	Cd and Pb interacted synergistically to inhibit photosynthesis and nitrogenase activity in *Anabaena inequalis*	(93)
	Zn and Pb interacted synergistically, but Hg and Ni interacted antagonistically, to Cd-induced mitotic delay in *Physarum polycephalum*	(94)
	Zn and Cd interacted synergistically to inhibit growth of the marine diatoms, *Thalassiosira pseudonana* and *Skeletonema tricornutum*; Zn interacted antagonistically to the toxicity of Cd to growth of *Skeletonema costatum*	(95)
Inorganic anions	Cd^{2+} was more inhibitory than was an equivalent concentration of Cd as $Cd(CN)_4^{2-}$ towards growth of a mixed microbiota from activated sludge	(96)
	Increasing the chlorinity decreased the uptake of Cd by the estuarine alga, *Chlorella salina*	(97)
	Chloride, at a level equivalent to that occurring in seawater, decreased the toxicity of Cd to mycelial growth of *Sepedonium* sp., *Oospora* sp., *Trichoderma viride*, *Aspergillus niger*, *Rhizopus stolonifer*, and *Scopulariopsis brevicaulis*	(21)

recreation." When considering "aquatic life," EPA limited the scope to animals and plants, including in this category the unicellular algae (*6-8*). As the adverse effects of toxicants on the microbiota, primarily on bacteria and fungi, were not considered when formulating these criteria, EPA ignored the "identifiable effects" of these toxicants on the numerous microbe-mediated ecologic processes.

Microorganisms in aquatic and terrestrial ecosystems are dynamically involved in many basic ecologic processes, such as the biogeochemical cycling of chemical elements, the mineralization of carbon, nitrogen, sulfur, and phosphorus needed to maintain the fertility of the biosphere, the formation of organic matter by chemo- and photosynthesis, and the decomposition of plant and animal wastes. The hindrance of these microbe-mediated ecologic processes by anthropogenic pollutants would greatly affect the quality of the biosphere (*14-16*), eventually adversely affecting human health and welfare. For example, microorganisms, primarily fungi and bacteria, are involved in the decomposition of organic

matter, such as complex animal and plant tissues and excretory products. In addition to being "Nature's sanitary engineers," microbial conversion of organic matter to inorganic materials (i.e., mineralization) is an important nutrient regeneration process in aquatic (*100*) and terrestrial (*101*) ecosystems. Although most natural ecosystems contain an abundant supply of carbon, nitrogen, sulfur and phosphorus, the major portion of these elements occurs as organic complexes that, as such, are unavailable for uptake by the phytobiota (*102*). Reductions in the mineralization activities of microbes would initially affect the primary producer level, with plant growth being limited. As plants are the basic components of all food chains and webs, such perturbations in plant growth would hinder the population dynamics of herbivores, carnivores and omnivores, including human beings. Thus, an adverse effect on a microbe-mediated ecologic process such as mineralization would, by a "domino effect," eventually impinge on the continued health and welfare of human beings.

Microbes are sensitive to most pollutants (*14-17*), and an inhibition of microbial activity is accompanied by reductions in the ecologic processes that they perform. The adverse effects of toxicants on microbe-mediated ecologic processes have not, as yet, been incorporated into the formulations for computing criteria and standards of environmental risks (*18, 103*). For example, although Cd adversely affects many microbe-mediated ecologic processes (Table 5), EPA did not consider these processes when formulating the Water Quality Criteria for this metal (*104*) or for other toxicants (*6-8*). The need to examine environments in a "holistic framework," including microbe-mediated ecologic processes, has been noted as a goal in the 1980s for environmental analysts (*105*).

It is difficult to understand the failure of environmental policy analysts and policy makers to consider the adverse effects of toxicants on microbe-mediated ecologic processes when formulating criteria such as the Water Quality Criteria and standards such as the National Secondary Air Quality Standards. The failure may be due to the inability to compare easily, and, thus, to evaluate and incorporate into the existing methodologies used to compute environmental criteria and standards the extent of damage by a toxicant to an ecologic process in different types of ecosystems. More probably, environmental toxicology has simply not developed to the point where the need to consider an adverse affect on an ecologic process is appreciated. It has been stated that aquatic toxicologists have only begun to address the "ecological effect" of toxicants (*106*).

Ecologic Dose Fifty Percent (EcD_{50})

The extent of pollutant damage to some microbe-mediated ecologic processes can be measured effectively in the laboratory. For example, heavy metals have been shown to interfere with several microbe-mediated ecologic processes, such as the biogeochemical cycling of nitrogen (*115, 122-135*), sulfur (*107*), phosphorus (*108, 133, 134*), and carbon (*108, 109, 111-115, 129, 136-138*); the decomposition of plant litter (*50, 109, 110, 117-119, 133, 139*); photosynthesis (*83, 92, 115, 121, 140*); and enzymatic activities (*11, 119, 131-134, 141, 142*). As these adverse effects on ecologic processes can be quantified, it is suggested that a formulation be derived, similar to the LD_{50} (i.e., the dose that is lethal to 50% of the exposed population) which has been used extensively to compute standards for exposures of human beings and the general biota to toxicants (*143*), to allow environmental analysts and policy-makers easily to compute the extent of damage by a toxicant to a microbe-mediated ecologic process and to compare the extent of damage by the same toxicant to a common ecologic process in different types of ecosystems. Such a formulation, termed the "ecologic dose fifty percent" (EcD_{50}) and defined as the dose of a toxicant that decreases a specific microbe-mediated ecologic process by 50% (other percentages of decrease could also be used), would permit regulatory agencies to incorporate such data into the existing methodologies used in establishing environmental criteria and standards (*18, 103*).

The EcD_{50} can be determined in a manner similar to that used for the LD_{50}, in which a population, or in the case of the EcD_{50}, a microbe-mediated ecologic process, is exposed to progressively increasing levels of a toxicant. The resulting data, when plotted as percent mortality for the LD_{50} or as percent inhibition for the EcD_{50} versus the concentration of toxicant, should approximate a broad S-shaped curve from which the LD_{50} (*144*) or the EcD_{50} can be computed. The LD_{50} test, which was developed initially in 1927 for the biological standardization of hazardous drugs, has been incorporated into the routine toxicological protocol for other classes of chemicals and now is part of practically all Federal guidelines that regulate the toxicological testing of chemicals (*145*). Currently, toxicologists determine LD_{50} values of environmental chemicals for plant and animal species representative of specific ecosystems, and then, environmental policy-makers utilize the LD_{50} values of the most sensitive species as the bases on which to formulate criteria. Similarly, EcD_{50} values could be computed for different ecologic processes stressed by a common pollutant, and the EcD_{50} value of the most sensitive microbe-mediated eco-

Table 5. Effects of cadmium on some microbe-mediated ecologic processes in aquatic and terrestrial ecosystems.

Ecologic process	Comments	Reference
Soil enzymatic activity	25 μmole Cd/g soil inhibited arylsulfatase activity	(107)
	25 μmole Cd/g soil inhibited the activities of acid and alkaline phosphatases	(108)
Carbon mineralization	10 ppm Cd inhibited soil respiration	(109)
	Soil respiration was decreased by addition of 10 ppm Cd + 1000 ppm Zn	(110)
	Starch decomposition and soil respiration were reduced in a spruce needle mor contaminated with Cu, Zn, Pb, and Cd emitted from a brass foundry	(111)
	Carbon mineralization in soil was inhibited by 100 ppm Cd	(112)
	1000 ppm Cd extended the lag phase of glucose degradation in soil; no synergistic interaction was noted between 1000 ppm Cd and up to 10,000 ppm Zn or simulated acid rain causing a reduction in soil pH to 2.8 or 3.2 to glucose degradation	(113, 114)
	10 ppm Cd inhibited glucose oxidation in Chesapeake Bay water and sediment	(115)
Litter decomposition	Rates of decomposition of spruce needle litter obtained from sites near metal-processing industries emitting Cu, Zn, Ni, and Cd were reduced as compared to litter obtained from nonpolluted sites	(116)
	Decomposition rates of leaf litter from *Quercus velutina*, *Smilacina stellata*, and *Populus tremuloides* were reduced in a site contaminated with Cd, Zn, Pb and Cu	(117)
	Decomposition rates of litter consisting of leaves from *Sassafras albidum*, *Quercus prinus*, and *Quercus rubra* and contaminated with Cd, Cu, Fe, Pb and Zn were lower as compared to similar litter from a nonpolluted site	(118)
	1000 μg Cd/g soil inhibited decomposition of a Douglas-fir needle litter	(119)
	Decomposition of leaves of *Pinus taeda*, *Sassafras albidum*, *Quercus nigra*, *Quercus laurifolia*, *Prunus americana*, and *Acer rubrum* was decreased in a freshwater ecosystem amended with 5 μg Cd/L	(120)
Microbial photosynthesis	0.1 mg Cd/L reduced photosynthesis of a brackish water phytoplankton community	(92)
	25 ppm Cd inhibited photosynthesis in Chesapeake Bay water	(115)
	100 *nM* Cd inhibited growth of a marine phytoplankton community	(121)
	10^{-6} *M* Cd inhibited photosynthesis of a freshwater phytoplankton community consisting mainly of diatoms	(85)
Nitrogen cycle		
Denitrification	Denitrification by the indigenous microbiota was reduced by 100 μg Cd/g soil	(122)
Nitrification	0.01 to 0.04*M* Cd inhibited nitrification in soil	(123)
	Nitrification was reduced by Cd concentrations up to 400 μg Cd/g soil but was enhanced at levels from 400 to 2,500 μg Cd/g soil	(124)
	5 μmole Cd/g soil inhibited nitrification	(125)
	500 ppm Cd reduced nitrification in soil; at 1000 ppm Cd, nitrite accumulation was evident	(126)
	Nitrification was reduced in Chesapeake Bay water amended with 100 ppm Cd	(115)
Nitrogen fixation	18μ*M* Cd inhibited nitrogen fixation by soybean nodules containing *Rhizobium japonicum*	(127)
	Nitrogen fixation of a Douglas-fir needle litter was decreased by amendments of 5 *mM* Cd/g soil	(85)

logic process could be used to formulate criteria (*18*, *103*).

The EcD_{50} has three distinct advantages over the LD_{50}. First, LD_{50} values are for populations of single species, which usually are of uniform size, age, physiological and genetic constitution, etc., and, therefore, do not display the heterogeneity of natural populations (*146*). Standards based on such single species populations may not, therefore, adequately protect the biosphere. Conversely, most microbe-mediated ecologic processes are controlled by the combined metabolism of different species of bacteria and fungi, and thus, an EcD_{50} value reflects the combined response of a variety of populations to a stress. Second, the species selected to be assayed in LD_{50} tests may be of limited importance to a natural ecosystem, and when toxic effects are noted, determinations must then be made as to whether the presence of the species is critical to the continued functioning of the ecosystem. However, a greater risk and perturbation to the functioning of an ecosystem would be the inhibition or removal of an entire functional group, such as decomposers, nitrogen fixers, or primary producers (*147*). The determination of EcD_{50} values would, therefore, have more relevance than would LD_{50} values for predicting the continued functioning of stressed ecosystems. Third, with the LD_{50} test, a direct comparison be-

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tween the sensitivities to a toxicant of species that dwell in different ecosystems is not always possible. For example, it may be necessary to compare the sensitivity to a pollutant of a marine and a fresh water fish. These comparisons are difficult, as the possible effects resulting from the differences in the environments are confounded by differences in the test species. However, as most microbe-mediated ecologic processes are common to all ecosystems, a reduction in a process in one ecosystem by a toxicant can easily be compared with a similar reduction by that toxicant in the same ecologic process but in a different ecosystem. For example, the level of toxicant inducing a 50% reduction in carbon mineralization in fresh waters can be compared to the level of that toxicant evoking an equivalent reduction in carbon mineralization in marine waters (*18, 103*).

Although it is suggested that the EcD_{50} concept be incorporated into regulatory decision-making, it is recognized that this concept needs to be more fully analyzed and developed by the scientific community. For example, a 50% reduction in a basic ecologic process may be a value that is too extreme for the continued functioning of a perturbed ecosystem and, perhaps, an EcD_{25} or EcD_{10} would be more suitable. Also, the EcD_{50} of a specific ecologic process-pollutant interaction should not be viewed as a constant value, as the EcD_{50} value may depend on the length of exposure and on the properties of the test ecosystem. For example, an EcD_{50} value determined after 2 days of exposure, during which a temporary lag may occur in the ecologic process being studied, may be entirely different if determined after 2 weeks of exposure, during which time the stressed populations may have adapted to the toxicant or may have been replaced by populations having comparable metabolic capabilities (*113, 114, 126, 148*). An EcD_{50} value for an ecologic process-pollutant interaction may be different for hard fresh waters than for soft fresh waters. These "problems" are not unique to the EcD_{50} but also apply to the LD_{50}, and it is common for toxicologists to determine an LD_{25} or LD_{100} or to determine an LD_{50} after 24, 48 or 96 hr or even after 2 weeks of exposure. Although not often emphasized, the LD_{50} is also not a constant but is dependent on or, at least, influenced by species, age, weight, sex, genetic constitution, health, diet, method of exposure, ambient temperature, seasonal variation, etc. (*145*).

Another aspect that will require considerable development is the application of appropriate statistical designs and analyses to the ecologic data used for calculating EcD_{50} values. This problem is also not unique to the EcD_{50} concept, as the appropriate statistics for LD_{50} data and risk levels of carcinogens and other environmental chemicals are still being debated (*19, 149-158*). The accumulation of sufficient data and numerous attempts to apply the EcD_{50} concept should, with the aid of statisticians, resolve this problem.

The EcD_{50} concept can be applied to many areas of environmental toxicology and is not limited to the Water Quality Criteria. There have been few legislative or regulatory initiatives designed to protect soil as an ecosystem, even though pollutants may cause serious adverse effects on microbe-mediated ecologic processes in terrestrial ecosystems. Consequently, the implementation of EcD_{50} values in risk analysis of aquatic ecosystems should have immediate application to terrestrial ecosystems similarly stressed by pollutants and, thus, may result in the establishment of Soil Quality Criteria.

Conclusions

Cairns (*146*), in discussing future needs in the biologic assessment of pollutants, mentions two concepts: "pollutant realism" and "environmental realism." Pollutant realism is attained when those characteristics of the test compound that exist in the natural environment are incorporated into the laboratory test system. As EPA has begun to recognize that the physicochemical properties of the recipient environment influence the toxicity of a pollutant to the indigenous biota, such abiotic factors should be routinely considered when formulating environmental criteria and standards. However, at present, the data base for such interactions is insufficient, and laboratory tests using animals and plants are too tedious and expensive. As the influence of abiotic factors on the response of microbes to pollutants is similar to that exhibited by more complex systems (i.e., plants and animals), it is suggested that microbial assays be used initially to identify those abiotic factors that most influence the toxicity of the various pollutants. Once these factors have been defined, additional studies should be performed with these factors using macrobiotic species representative of the stressed ecosystems and then criteria and standards formulated. Environmental realism is attained when the tests account for all aspects of the ecosystem, including those ecologic processes controlled by microbial activities. Microbe-mediated ecologic processes are critical to the continued functioning of the biosphere, and some of the environmentally oriented Federal statutes, such as TSCA, specify that adverse effects of pollutants on the environment must be determined. Thus, it is also recommended that these ecologic events be considered in the regulatory process, and it is further sug-

gested that an EcD_{50} formulation would be a useful tool to simplify their incorporation.

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