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David M. Whitacre
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Foreword

International concern in scientific, industrial, and governmental communities over traces of xenobiotics in foods and in both abiotic and biotic environments has justified the present triumvirate of specialized publications in this field: comprehensive reviews, rapidly published research papers and progress reports, and archival documentations. These three international publications are integrated and scheduled to provide the coherency essential for nonduplicative and current progress in a field as dynamic and complex as environmental contamination and toxicology. This series is reserved exclusively for the diversified literature on “toxic” chemicals in our food, our feeds, our homes, recreational and working surroundings, our domestic animals, our wildlife, and ourselves. Tremendous efforts worldwide have been mobilized to evaluate the nature, presence, magnitude, fate, and toxicology of the chemicals loosed upon the Earth. Among the sequelae of this broad new emphasis is an undeniable need for an articulated set of authoritative publications, where one can find the latest important world literature produced by these emerging areas of science together with documentation of pertinent ancillary legislation.

Research directors and legislative or administrative advisers do not have the time to scan the escalating number of technical publications that may contain articles important to current responsibility. Rather, these individuals need the background provided by detailed reviews and the assurance that the latest information is made available to them, all with minimal literature searching. Similarly, the scientist assigned or attracted to a new problem is required to glean all literature pertinent to the task, to publish new developments or important new experimental details quickly, to inform others of findings that might alter their own efforts, and eventually to publish all his/her supporting data and conclusions for archival purposes.

In the fields of environmental contamination and toxicology, the sum of these concerns and responsibilities is decisively addressed by the uniform, encompassing, and timely publication format of the Springer triumvirate:

Reviews of Environmental Contamination and Toxicology [Vol. 1 through 97 (1962–1986) as Residue Reviews] for detailed review of articles concerned with any aspects of chemical contaminants, including pesticides, in the total environment with toxicological considerations and consequences.

- *Bulletin of Environmental Contamination and Toxicology* (Vol. 1 in 1966) for rapid publication of short reports of significant advances and discoveries in the fields of air, soil, water, and food contamination and pollution as well as methodology and other disciplines concerned with the introduction, presence, and effects of toxicants in the total environment.
- *Archives of Environmental Contamination and Toxicology* (Vol. 1 in 1973) for important complete articles emphasizing and describing original experimental or theoretical research work pertaining to the scientific aspects of chemical contaminants in the environment.

Manuscripts for *Reviews* and the *Archives* are in identical formats and are peer reviewed by scientists in the field for adequacy and value; manuscripts for the *Bulletin* are also reviewed but are published by photo-offset from camera-ready copy to provide the latest results with minimum delay. The individual editors of these three publications comprise the joint Coordinating Board of Editors with referral within the board of manuscripts submitted to one publication but deemed by major emphasis or length more suitable for one of the others.

Coordinating Board of Editors

Preface

The role of *Reviews* is to publish detailed scientific review articles on all aspects of environmental contamination and associated toxicological consequences. Such articles facilitate the often complex task of accessing and interpreting cogent scientific data within the confines of one or more closely related research fields.

In the nearly 50 years since *Reviews of Environmental Contamination and Toxicology* (formerly *Residue Reviews*) was first published, the number, scope, and complexity of environmental pollution incidents have grown unabated. During this entire period, the emphasis has been on publishing articles that address the presence and toxicity of environmental contaminants. New research is published each year on a myriad of environmental pollution issues faced by people worldwide. This fact, and the routine discovery and reporting of new environmental contamination cases, creates an increasingly important function for *Reviews*.

The staggering volume of scientific literature demands remedy by which data can be synthesized and made available to readers in an abridged form. *Reviews* addresses this need and provides detailed reviews worldwide to key scientists and science or policy administrators, whether employed by government, universities, or the private sector.

There is a panoply of environmental issues and concerns on which many scientists have focused their research in past years. The scope of this list is quite broad, encompassing environmental events globally that affect marine and terrestrial ecosystems; biotic and abiotic environments; impacts on plants, humans, and wildlife; and pollutants, both chemical and radioactive; as well as the ravages of environmental disease in virtually all environmental media (soil, water, air). New or enhanced safety and environmental concerns have emerged in the last decade to be added to incidents covered by the media, studied by scientists, and addressed by governmental and private institutions. Among these are events so striking that they are creating a paradigm shift. Two in particular are at the center of ever-increasing media as well as scientific attention: bioterrorism and global warming. Unfortunately, these very worrisome issues are now superimposed on the already extensive list of ongoing environmental challenges.

The ultimate role of publishing scientific research is to enhance understanding of the environment in ways that allow the public to be better informed. The term “informed public” as used by Thomas Jefferson in the age of enlightenment conveyed the thought of soundness and good judgment. In the modern sense, being “well informed” has the narrower meaning of having access to sufficient information. Because the public still gets most of its information on science and technology from TV news and reports, the role for scientists as interpreters and brokers of scientific information to the public will grow rather than diminish. Environmentalism is the newest global political force, resulting in the emergence of multinational consortia to control pollution and the evolution of the environmental ethic. Will the new politics of the twenty-first century involve a consortium of technologists and environmentalists, or a progressive confrontation? These matters are of genuine concern to governmental agencies and legislative bodies around the world.

For those who make the decisions about how our planet is managed, there is an ongoing need for continual surveillance and intelligent controls to avoid endangering the environment, public health, and wildlife. Ensuring safety-in-use of the many chemicals involved in our highly industrialized culture is a dynamic challenge, for the old, established materials are continually being displaced by newly developed molecules more acceptable to federal and state regulatory agencies, public health officials, and environmentalists.

Reviews publishes synoptic articles designed to treat the presence, fate, and, if possible, the safety of xenobiotics in any segment of the environment. These reviews can be either general or specific but properly lie in the domains of analytical chemistry and its methodology, biochemistry, human and animal medicine, legislation, pharmacology, physiology, toxicology, and regulation. Certain affairs in food technology concerned specifically with pesticide and other food-additive problems may also be appropriate.

Because manuscripts are published in the order in which they are received in final form, it may seem that some important aspects have been neglected at times. However, these apparent omissions are recognized, and pertinent manuscripts are likely in preparation or planned. The field is so very large and the interests in it are so varied that the editor and the editorial board earnestly solicit authors and suggestions of underrepresented topics to make this international book series yet more useful and worthwhile.

Justification for the preparation of any review for this book series is that it deals with some aspect of the many real problems arising from the presence of foreign chemicals in our surroundings. Thus, manuscripts may encompass case studies from any country. Food additives, including pesticides, or their metabolites that may persist into human food and animal feeds are within this scope. Additionally, chemical contamination in any manner of air, water, soil, or plant or animal life is within these objectives and their purview.

Manuscripts are often contributed by invitation. However, nominations for new topics or topics in areas that are rapidly advancing are welcome. Preliminary communication with the editor is recommended before volunteered review manuscripts are submitted.

Summerfield, North Carolina David M. Whitacre

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The Water Quality of Izmir Bay: A Case Study

Filiz Kucuksezgin

Contents

1 Introduction

Marine pollution refers to direct or indirect contamination of the marine environment (including estuaries) by humans. Such marine pollution may result from substances or from energy and may produce harm to living organisms, hazards to human health, hindrances to marine activities such as fishing, impairment of seawater quality, and reduction of amenities. Contaminants are transported from land to sea by streams and rivers, direct runoff, or point discharges. Contaminants are partly retained in the water and in the sediments of estuaries, bays, beaches, or open coastal waters and are partly transported through these boundary areas to the open ocean. Moreover, coastal waters are not only more polluted than is the open ocean but also offer a significantly higher probability of exposure to the world's most important fisheries and recreational areas (Magos 1990).

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A group of chemicals known as priority pollutants has been identified by the United States Environmental Protection Agency (USEPA) to present health or environmental risks at certain water concentrations. The priority pollutant list includes both organic compounds, such as chloroform and toluene, and inorganic chemicals, such as heavy metals (e.g., copper, zinc, nickel, chromium, and lead) (USEPA 1976, 1986).

Among the myriad of organic and inorganic substances released into aquatic ecosystems, heavy metals have received considerable attention because of their toxicity and potential bioaccumulation in many aquatic species (Gupta and Mathur 1983). The presence of heavy metals in aquatic ecosystems is the result of two main sources of contamination: natural processes or natural occurring deposits and anthropogenic activities. The main source of heavy metal contamination and the threat of heavy metal pollution to life forms is invariably the result of anthropogenic activities (Kennish 1992). In the marine environment, toxic metals are potentially accumulated in sediments and in marine organisms and subsequently transferred to man through the food chain. Thus, it has become increasingly important to determine and assess levels of heavy metal concentrations in marine organisms for several purposes. Knowing such information has implications for human nutrition and safety (fish are a main source of human dietary protein), utility for use as indicators of marine pollution, and helps in monitoring for point-source metal pollutants (Sadiq et al. 1995).

There are many sources of toxic metal pollutants that may affect fish and other species; some of these are as follows: accidental spills of chemical waste, periodic precipitation of contaminated airborne pollutants, discharge of industrial or sewage effluents, agricultural drainage, domestic wastewater, and gasoline disposal from fishing vessels (Handy 1994; Jent et al. 1998). Fish have also been contaminated by heavy metals that entered water from fertilizers that contain heavy metals (Chaisemartin 1983).

Nutrient oligotrophic or eutrophic conditions have been characterized as the principal factors affecting the marine ecosystem (Dugdale 1967; Ryther and Dunstan 1971). Certain past studies have associated oligotrophy with the absence of measurable concentrations of a nutrient (Ignatiades et al. 1992; Kucuksezgin et al. 1995) and have defined eutrophication as a qualitative parameter that indicates nutrient or organic matter enrichment from external sources that produce high biological productivity (Ignatiades et al. 1992).

Pollution of the sea by organic toxicants, such as petroleum hydrocarbons, occurs mainly through marine operations, land-based discharges, and atmospheric and natural inputs (IMCO 1977; GESAMP 1993; Laws 2000). The total input of petroleum to the oceans through human activities and sources, such as atmospheric fallout and natural seepage, is estimated at 2.37–106 t year^{-1} (Kennish 1997). Of this total, about 65.2% is discharged through municipal and industrial wastes, urban and river runoff, oceanic dumping, and atmospheric fallout; 26.2% derives from discharges during transportation, dry docking, tanker accidents, de-blasting, etc., and the remaining 8.5% of discharges comes from fixed installations such as coastal refineries, offshore production facilities, and marine terminals (GESAMP 1993).

Though a considerable fraction of petroleum hydrocarbons entering the marine environment is removed by evaporation, a portion gets distributed in water, accumulates in sediment, and is transferred to biota.

Pesticides, which are primarily organic in nature, are significant pollutants and reach aquatic ecosystems by direct application, spray drift, aerial spraying, erosion and runoff from agricultural or residential land, or by discharge of effluents from factories and sewage (Beitz et al. 1994; Guidotti et al. 2000). Organochlorine pesticides (OCPs) are no longer widely used because they possess several disadvantages that include environmental persistence, bioaccumulation, and toxic effects on the nervous system (Hellawell 1988). Their entry into ecosystems not only may affect humans but also may adversely affect many non-target organisms including fish and birds (Ayas et al. 1997; Grasman and Fox 2001; Turgut and Fomin 2002). Another major negative characteristic of the organochlorines is that they are known to undergo biomagnification via the food chain (Turgut and Fomin 2002).

The objective of this chapter is to review the available literature that pertains to the level of pollution in the Izmir Bay by nutrients, trace metals, and anthropogenic-derived organic compounds. In addition, I will endeavor to assess whether any conclusion, either regional or otherwise, can be drawn from this assessment.

2 General Aspects of Pollution in the Izmir Bay

Izmir Bay is one of the great natural bays of the Mediterranean. The main conurbation around the bay is the Izmir Metropolitan Municipality, covering 88,000 ha and having a population of nearly 3 million inhabitants. Izmir is an important industrial and commercial center and a cultural focal point. The bay has a total surface area of over 500 km^2, a water capacity of 11.5 billion m^3, a total length of 64 km, and the bay opens into the Aegean Sea. The depth of water in the Outer Bay is about 70 m and decreases toward the Inner Bay. The climate of the bay is relatively mild, having an annual average temperature of 17°C. The bay has been divided into three sections (Outer, Middle, and Inner) according to the physical characteristics of the different water masses.

The Outer Bay extends from Kokola point to the mouth of the bay (Fig. 1). The water volume of the Outer Bay is 1×10^{10} m^3, and it has an average water depth of about 49 m. The surface area of the Outer Bay is approximately 417 km^2. Pollution in the Outer Bay is not significant, and this part of the bay is relatively clean according to most pollution indicators. In fact, a wildlife conservation area (the Foca Pilot Monk Seal Conservation Area) is situated at the entrance of the Outer Bay.

The Gediz River, which flows into the Outer Bay, is the second biggest river along the Aegean Coast. There is a dense population along the Gediz River, and the surrounding areas include extensive agricultural lands and numerous manufacturing, food, and chemical industries. The Gediz River drains a basin that is 15,616 km^2 in area and has an average annual discharge rate of 40–70 $m^3\ s^{-1}$ (EIE 1984). The seaward fringe of the Gediz Delta is an important nature reserve and has recently been designated as a RAMSAR (a convention on wetlands of international importance,

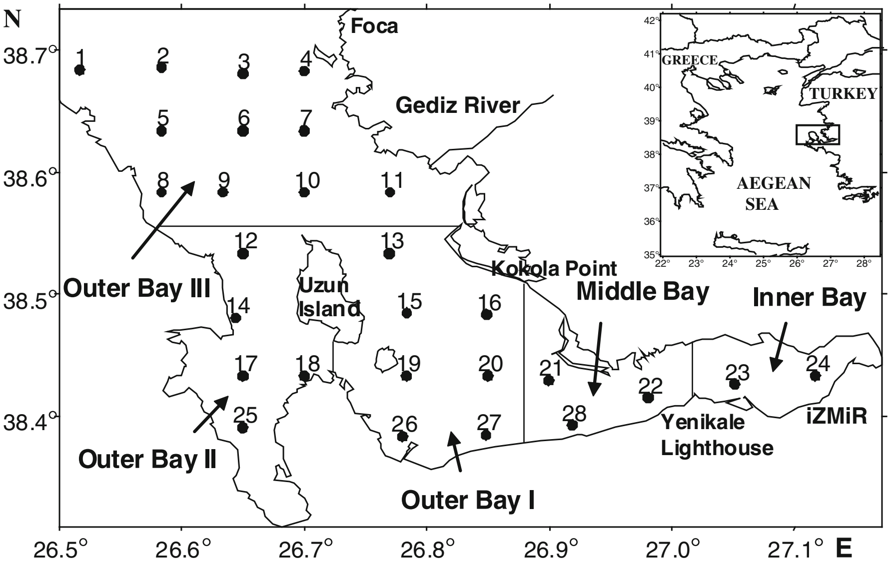

Fig. 1 Key geographic features of different sections of Izmir Bay

especially as a waterfowl habitat) site to protect rare bird species (MEF 1995, 2000). Originally, the area received excess water from the Gediz River for much of the year, but since 1990, with restrictions on irrigation releases, the reserve suffers from water shortages. The Gediz Delta comprises an extensive coastal wetland that has bays, salt and freshwater marshes, large saltpans, and four lagoons that have formed at the mouth of the Gediz River. This river, of course, transports wastes; to prevent soil particles from settling out before reaching the sea and to provide sufficient dilution to avoid in-stream environmental harm, the flow rate of the river must have sufficient velocity to keep organic compounds or metals that are adsorbed to particulates, in suspension.

The Middle Bay extends from the Yenikale lighthouse to Kokola point. The water volume of the Middle Bay is 9×10^8 m^3 and has an average water depth of about 16 m. The surface area of the Middle Bay is approximately 57 km^2. The water of the Middle Bay retains pollutant concentrations intermediate between those of the Outer and Inner Bays, which is a clear indication that pollution in the bay does spread. Because of the shallow nature of the Inner and Middle Bays, water exchange and self-purification capacities are very limited.

A 13-m-deep sill, the Yenikale Strait, separates the Middle Bay from the Inner Bay. The Inner Bay is heavily polluted by nutrients and organic material (UNEP 1993). The water volume of the Inner Bay is 6×10^8 m^3, and it has an average water depth of about 7 m (range between 0 and 20 m). The Inner Bay is heavily polluted by organic material and nutrients; however, metal concentrations were not high enough to indicate heavy metal pollution. The northern part of the Inner Bay had been filled by silting deposition from the Gediz River, released west of Karsıyaka. Therefore, the Izmir Harbor has encountered a shoaling problem, and the entrance to the Gediz River has migrated toward the Outer Bay. The depth of the bay, going from the onshore to the offshore area, increases steeply so that incoming and outgoing ships must follow a shallow 10-m-deep channel (between the old Gediz Delta and Narlıdere) (UNEP 1993).

The main industries in the region include food processing, beverage manufacturing and bottling, tanneries, oil, soap and paint production, chemical manufacturing, paper and pulp factories, textile production, and metal and timber processing. About 6000 industrial establishments are registered with the Chamber of Industry in Izmir. However, there are many other establishments that are unregistered. The main sources of pollution in the bay derive from domestic and industrial effluents, which account for 50% of the observed organic pollution.

Nutrient oligotrophic or eutrophic conditions have been characterized as the principal factors affecting the local marine ecosystem (Ryther and Dunstan 1971). Results of several past studies have associated oligotrophy with the absence of measurable concentrations of a nutrient (Ignatiades et al. 1992; Kucuksezgin et al. 1995), and eutrophication has been defined as a qualitative parameter in which there is nutrient or organic matter enrichment from external sources that results in high biological productivity (Ignatiades et al. 1992). The contribution of nutrients to eutrophication from sediments in the Inner Bay was reported by Ozkan et al. (2008).

The Inner Bay is heavily polluted by nutrients and other organic materials, but metal concentrations were not so high as to label the waters as being polluted by heavy metals. Industrial fluxes of Cr (chromium), Cd (cadmium), and Hg (mercury) to the bay were 6700, 20 and 70 kg year^{-1}, respectively. Data are not available on fluxes of heavy metals that result from domestic discharges. Amounts equal to 105,000 m^3 day^{-1} of industrial and 308,000 m^3 day^{-1} of domestic wastewater were discharged to the bay without significant treatment (UNEP 1993) until 2000. In early 2000, a wastewater treatment plant (WTP) began operation to treat domestic and industrial wastes. Eutrophication of the Inner Bay is a serious problem throughout the year and red tide events are becoming more frequent (UNEP 1993; Institute of Marine Sciences and Technology (IMST)-070/A 1991; Kontas et al. 2004).

Heavy metals, as defined by Nieboer and Richardson (1981), are normal constituents of the marine environment. At least 10 are known to be essential to marine organisms: Fe (iron), Cu (copper), Zn (zinc), Co (cobalt), Mn (manganese), Cr (chromium), Mo (molybdenum), V (vanadium), Se (selenium), and Ni (nickel). These metals always function in combination with organic molecules, usually proteins. Metals occur normally at low concentrations yet are capable of exerting considerable biological effects even at such levels (Rainbow 1992). All metals are toxic above some bioavailable threshold level. Ag (silver), Hg, Cu, Cd, and Pb (lead) are particularly toxic. The elucidation of the comparative pollution of aquatic environments by heavy metals is possible through analysis of water, sediments, and members of indigenous biota, i.e., biomonitors (Phillips and Rainbow 1993).

Sediments are composite materials consisting of inorganic components, mineral particulates, and organic matter in various stages of decomposition. The chemical composition of sediments is considered to be a sensitive indicator for both natural and anthropogenic contaminants in the marine environment (Salamons 1995;

Calmano et al. 1996). Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous organic contaminants of marine coastal sediments (NOAA 1988; Raoux et al. 1999). PAHs show a marked hydrophobic character, resistance to biodegradation (Soclo et al. 2000), and produce adverse effects on animal health (carcinogenic and/or mutagenic activity) (Henner et al. 1997; Singh et al. 1998) and on ecosystems (Varanasi et al. 1985; Long et al. 1995).

Metal pollution of the sea is less visible and direct than other types of marine pollution, but its effects on marine ecosystems and humans are intensive and very extensive. As an indirect measure of the abundance and availability of metals in the marine environment, the bioaccumulation of metals by the tissues of marine organisms is routinely studied. Such bioaccumulation studies have led to the adoption of the bioindicator concept (Langston and Spence 1995), and fish are widely used as bioindicators of marine pollution by metals (Evans et al. 1993). Several studies have been conducted on the concentrations of nutrients and heavy metals that exist in Izmir Bay (Buyukisik 1986; Buyukisik and Erbil 1987; Demirkurt et al. 1990; Parlak and Demirkurt 1990; Kucuksezgin and Balci 1994; Parlak et al. 1994; Balci et al. 1995; Kucuksezgin 1996; Buyukisik et al. 1997; Kucuksezgin et al. 2002, 2004, 2005, 2006, 2008; Sunlu 2002, 2004, 2006; Yucel-Gier et al. 2007), and these will be reviewed below.

3 The Water Quality of Izmir Bay

3.1 *Nutrients*

Eutrophication problems have occurred in many coastal areas. Such problems derive from conditions characterized as follows: areas that are enclosed or semi-enclosed and have poor water exchange are affected by urban and industrial wastewaters and/or receive nutrient inputs from rivers and urban activities. There is an apparent eutrophication problem in such bays due in large measure to inputs of nutrients and organic matter into the shallow zone that then has limited exchange with the open sea. Much of the polluting nutrients and organic materials derive from domestic sewage from high population density, coastal agglomeration, and tourism. The UWWT (The Urban Waste Water Treatment) Directive defines eutrophication as “the enrichment of water by nutrients, especially compounds of nitrogen and/or phosphorus, causing an accelerated growth of algae and higher forms of plant life to produce an undesirable disturbance to the balance of organisms present in the water and to the quality of the water concerned” (UNEP 2007).

The water quality in the Izmir Bay has been significantly affected by past and continuing coastal and industrial development. Nutrient concentrations in the bay have been dramatically changed by the conversion of natural creeks and freshwater inputs to WTP from the large and rapidly expanding Izmir metropolitan area. In this review, I evaluate the nutrient loadings input to Izmir Bay during the period 1996–2008; such inputs to the bay have come from the Gediz River, from the atmosphere, and from the natural creeks that empty into it. The water quality of Izmir

Bay has perhaps been most significantly affected by the Gediz River, which drains surrounding agricultural and urban areas.

The average nutrient concentrations from all depths in the Outer Bay ranged between 0.01 and 0.22, 0.10 and 1.8, 0.10 and 0.98, 0.30 and 5.9 μM for orthophosphate-phosphorus (o-PO$_4$-P), (nitrate+nitrite)-nitrogen (TNO$_x$-N), ammonium (NH$_4$-N), and reactive silicate (Si(OH)$_4$), respectively (Table 1).

Table 1 Range and year(s) found of several nutrients detected in Izmir Bay at different locations: phosphate (o-PO$_4$), total phosphate (T-PO$_4$), nitrate+nitrite (TNO$_x$), ammonium (NH$_4$), silicate (Si(OH)$_4$) (μM), and trophic index (TRIX)

	Period	Outer Bay	Middle–Inner Bays	References
o-PO$_4$	1996–1998	0.01–0.19	0.01–10	Kucuksezgin et al. (2006)
	2000	0.01–0.11	0.13–3.8	Kucuksezgin et al. (2006)
	2001	0.02–0.10	0.14–2.9	Kucuksezgin et al. (2006)
	2002	0.01–0.19	0.14–4.4	Kucuksezgin et al. (2006)
	2003	0.01–0.16	0.32–4.5	Kucuksezgin et al. (2006)
	2004	0.01–0.12	0.04–6.7	IMST-152 (2004)
	2005	0.01–0.16	0.12–5.0	IMST-152 (2005)
	2007	0.01–0.21	0.02–9.8	IMST-167 (2007)
	2008	0.01–0.22	0.01–4.3	Kucuksezgin (2009)
T-PO$_4$	2000	0.06–0.85	0.52–6.9	Kucuksezgin et al. (2006)
	2001	0.11–0.61	0.20–3.0	Kucuksezgin et al. (2006)
	2002	0.12–0.66	0.38–4.8	Kucuksezgin et al. (2006)
	2003	0.10–0.69	0.28–5.9	Kucuksezgin et al. (2006)
	2004	0.13–0.59	0.11–6.9	IMST-152 (2004)
	2005	0.08–0.89	0.22–8.8	IMST-152 (2005)
	2007	0.04–0.80	0.04–10.5	IMST-167 (2007)
	2008	0.04–0.82	0.04–4.6	Kucuksezgin (2009)
TNO$_x$	1996–1998	0.11–1.8	0.13–27	Kucuksezgin et al. (2006)
	2000	0.11–1.0	0.15–18	Kucuksezgin et al. (2006)
	2001	0.18–0.90	0.29–16	Kucuksezgin et al. (2006)
	2002	0.10–1.4	0.26–6.7	Kucuksezgin et al. (2006)
	2003	0.10–1.4	0.12–8.6	Kucuksezgin et al. (2006)
	2004	0.11–1.4	0.11–3.0	IMST-152 (2004)
	2005	0.11–1.0	0.11–5.9	IMST-152 (2005)
	2007	0.10–0.73	0.10–4.1	IMST-167 (2007)
	2008	0.10–0.85	0.12–4.1	Kucuksezgin (2009)
NH$_4$	1996–1998	0.10–0.96	0.10–21	Kucuksezgin et al. (2006)
	2000	0.10–0.69	0.13–34	Kucuksezgin et al. (2006)
	2001	0.10–0.79	0.11–50	Kucuksezgin et al. (2006)
	2002	0.10–0.68	0.10–6.7	Kucuksezgin et al. (2006)
	2003	0.10–0.69	0.21–2.4	Kucuksezgin et al. (2006)
	2004	0.10–0.83	0.10–4.5	IMST-152 (2004)
	2005	0.10–0.88	0.14–6.5	IMST-152 (2005)
	2007	0.10–0.85	0.10–9.2	IMST-167 (2007)
	2008	0.10–0.85	0.10–33	Kucuksezgin (2009)
Si(OH)$_4$	1996–1998	0.30–4.1	0.50–39	Kucuksezgin et al. (2006)
	2000	0.38–2.8	0.43–20	Kucuksezgin et al. (2006)
	2001	0.48–1.7	1.2–18	Kucuksezgin et al. (2006)
	2002	0.72–4.8	1.0–26	Kucuksezgin et al. (2006)

Table 1 (continued)

	Period	Outer Bay	Middle–Inner Bays	References
	2003	0.80–5.8	2.6–32	Kucuksezgin et al. (2006)
	2004	1.1–5.1	0.76–21	IMST-152 (2004)
	2005	0.60–5.9	0.92–15	IMST-152 (2005)
	2007	0.60–3.8	0.55–14	IMST-167 (2007)
	2008	0.72–2.7	0.87–8.8	Kucuksezgin (2009)
TRIX	2000	1.41–5.39	3.94–9.82	Kucuksezgin (2009)
	2001	1.67–5.30	4.22–9.54	Kucuksezgin (2009)
	2002	2.18–5.48	4.48–10.3	Kucuksezgin (2009)
	2003	0.49–5.92	1.64–7.72	Kucuksezgin (2009)
	2004	1.43–3.75	2.19–6.27	Kucuksezgin (2009)
	2005	0.75–3.47	2.31–5.93	Kucuksezgin (2009)
	2007	0.58–3.08	0.55–5.59	Kucuksezgin (2009)
	2008	0.98–4.41	2.09–6.60	Kucuksezgin (2009)

During the autumns and winters of the period from 1996 to 2003, TNO_x-N levels were generally higher than those in spring and summer. The *o*-PO_4-P concentrations were similar to those of nitrate+nitrite concentrations. Maximum values were recorded during autumn and winter, because during these cooler times of year, phytoplankton consume less of these nutrients. During the winters and springs, high chlorophyll-a concentrations were measured in the Outer Bay and resulted from inputs from the Gediz River (Kucuksezgin et al. 2006). The Gediz River is known to be under a contamination menace that comes from industrial waste sources, sewage, and agricultural activities. The annual Gediz River discharges amount to 295,000 t of total suspended solids and 4900 t of nitrogen. Amounts of pollutants discharged by the Gediz River are higher than the total amounts entering the bay from other streams (UNEP 1993).

In the Middle–Inner Bays, the ranges of nutrient concentrations were 0.01–10, 0.12–27, 0.10–50, 0.43–39 μM for *o*-PO_4-P, TNO_x-N, NH_4-N, and $[Si(OH)_4]$, respectively (Table 1). Concentrations were comparatively higher in the Middle and Inner Bays than they were in the outer part of the bay. The maximum levels of *o*-PO_4-P and TNO_x-N were observed during summer and autumn and were due to bacterial degradation of particulate organic matter in the Middle–Inner Bays. The observed phosphate concentrations in this part of the bay were higher than values observed in clean waters, a clear indication of the polluting role of domestic waste in Izmir Bay.

The transparency of seawater (secchi disk depth) decreases toward the Inner Bay and depends on the productivity and the amount of terrestrial solid materials in the water. It reached a maximum value (29 m) in the Outer Bay during the winters and autumns and a minimum value (0.3 m) in the Inner Bay during summers as a result of increases in primary production. The secchi disk depth, an indicator of relative primary production and pollution levels in the marine environment, was comparatively high and independent of season in the Outer Bay.

The mean atomic ratio of TNO_x to PO_4 ranges from 8.3 to 11 in the Outer Bay, while the range at stations in the Middle and Inner Bays is 1.6–6.7; the differences at the two sites result from different characteristics of the seawater. The water column in Izmir Bay has a two-layer temperature structure during the summer as a result of radiant heating of the surface water. In winter, the water column has a temperature that is almost homogenous due to surface cooling and vertical mixing induced by winds. Maximum temperature and salinity values were observed during the summer, and minimum salinities were found near freshwater inputs. The observed mean N:P ratio was significantly lower than the assimilatory optimal ratio (N:P=15:1), which was in conformity with Redfield's ratio (N:P=16:1) in the bay; nitrogen is the limiting element in the Middle–Inner Bays. Diatoms and dinoflagellates are observed all year around in the bay and are normally nitrogen limited (Kucuksezgin et al. 2005).

Table 2 shows typical phosphate and nitrate concentrations in different areas of the Mediterranean and in the Izmir Bay. Nutrient concentrations are always detectable in Izmir Bay, and the levels found enhance photosynthetic production to levels that exceed the natural level of the marine environment.

Table 2 Range of nutrient (μM) values detected in samples taken from different parts of Izmir Bay and selected parts of the eastern Mediterranean Sea

	NO_3–N	NH_4–N	*o*-PO_4–P
Chios[a]	1.40–3.50	–	0.44–1.08
Pagasitikos Gulf[b]	0.07–2.21	0.05–3.20	0.03–0.31
Pagasitikos Gulf[a]	0.06–2.57	0.22–1.64	0.01–0.28
Izmir Outer Bay	0.10–1.40	0.10–0.85	0.01–0.22
Izmir Middle–Inner Bays	0.10–18.0	0.10–50.0	0.01–9.80

[a] Neofitou and Klaoudatos (2008)
[b] Petihakis et al. (2005)

The trophic index (TRIX) is a numerical value assigned to the trophic levels of coastal waters (Vollenweider et al. 1998). TRIX was used in Izmir Bay to classify the trophic status of the waters according to Environment Law as it is applied in Turkey. The mean values of TRIX varied between 2.06 and 3.87 and 3.40 and 7.29 in the Outer and Middle–Inner Bays, respectively (Table 1). In the Inner Bay, these values exceeded 6 in 2000, 2001, and 2002, which is typical for eutrophic areas. By analyzing the mean TRIX values for the different parts of Izmir Bay, the Inner Bay was classified as "eutrophic," and in contrast, the Outer Bay was characterized as "no-high eutrophication risk," according to the established standards of Turkish legislation. The Turkish legislative standards also rely on the TRIX to define acceptable eutrophication levels for aquaculture activity, according to the following scale: TRIX < 4, no eutrophication risk; $4 \leq$ TRIX ≤ 6, high eutrophication risk; TRIX > 6, clearly eutrophic (excessive nutrients).

To determine if there was a temporal trend in the data, linear regression analyses were performed using STATISTICA software (Release 7). Prior to the regression analysis, assumptions of normality and homogeneity of variances were checked. When these assumptions were not fulfilled, the dependent variables, i.e., nutrient or metal concentrations, were log transformed for the analyses. The time-dependent slopes of the regressions, their standard errors, the determination coefficients, and

Table 3 Results from the regression analyses of nutrient and TRIX data for different parts of Izmir Bay: slope of the regression (standard error), coefficient, p level, and sample size (n)

Data	n	Slope of regression	Coefficient	p level
Outer Bay				
TNO_x	2123	−0.047 (0.002)	0.15	<0.001
PO_4	2125	−0.038 (0.003)	0.063	<0.001
NH_4	1265	0.024 (0.004)	0.025	n.s.
Si	2121	0.012 (0.002)	0.014	<0.001
TRIX	1109	0.045 (0.006)	0.046	n.s.
Middle–Inner Bays				
TNO_x	713	−0.119 (0.009)	0.19	<0.001
PO_4	737	0.021 (0.011)	0.005	n.s.
NH_4	648	−0.072 (0.009)	0.08	n.s.
Si	729	0.026 (0.007)	0.02	<0.001
TRIX	352	−0.031 (0.019)	0.007	n.s.

associated p levels for PO_4, TNO_x, and Si in all data sets in the Outer Bay are presented in Table 3. Significant negative trends were observed in PO_4 and TNO_x concentrations, whereas an increase was seen in Si between 1996 and 2009 in the outer part of the bay. There was no significant trend in ammonium concentrations or TRIX values in this region. In contrast to the Outer Bay, non-significant trends appeared in PO_4 and Si in the middle–inner parts of the bay. In this region, a negative trend was identified for TNO_x (Table 3).

In early 2000, as mentioned, a WTP began to treat domestic and industrial wastes that entered the bay. This plant treated about a 60% capacity between 2000 and 2001. Although the capacity of this wastewater plant is sufficient for removal of nitrogen from the processed waste, it is inadequate for removal of phosphate. This is in accordance with the decreasing N/P ratios observed during 2000–2001 (after water processing had started) in the Middle–Inner Bays. Eutrophication of the Inner Bay is a serious problem throughout the year and the frequency of red tide events is increasing (IMST-167 2007).

3.2 Trace Metals

Concentrations of a total of six trace metals were reported from environmental samples (sediment and biota) collected in the bay for each sub-region (Atgin et al. 2000; Kontas 2006; Kucuksezgin et al. 2004, 2006; IMST-152 2005; IMST-180 2008). The metals detected were Hg, Cd, Pb, Cr, Cu, and Zn. Table 4 provides a summary of the concentration ranges of heavy metals detected in sediment samples collected from 1995 to 2008. Similar to what occurred with the nutrients, the highest concentrations of metals were found in the middle and inner parts of the bay, which are the closest to the center of anthropogenic activities and the intensely industrialized areas (e.g., iron, paper and pulp factories, antifouling paint pollution, chlorine-alkali plants, chemical production, textile industries, metal, food, and timber processing,

Table 4 The range of heavy metal levels found in sediments from different parts of Izmir Bay (μg g^{-1} dwt[a])

Period	Hg	Cd	Pb	Cr	Cu	Zn	References
Outer Bay							
1995	–	0.16–0.26	14–41	64–210	24–34	37–99	Atgin et al. (2000)
1996	0.20–1.89	–	–	–	–	–	Kontas (2006)
1997	0.19–0.96	0.07–0.25	6.7–70	19–157	2.2–34	14–134	Kucuksezgin et al. (2006)
1998	0.19–0.70	0.03–0.33	31–94	52–157	10–39	31–117	Kucuksezgin et al. (2006)
2000	0.26–0.99	0.009–0.09	46–76	41–172	7.9–34	28–110	Kucuksezgin et al. (2006)
2001	0.41–0.62	0.03–0.05	25–73	38–199	4.1–31	20–94	Kucuksezgin et al. (2006)
2002	0.10–0.28	0.01–0.07	14–76	40–149	2.6–36	20–167	Kucuksezgin et al. (2006)
2004	0.11–0.29	0.02–0.04	27–76	64–168	14–36	34–118	Kucuksezgin et al. (2004)
2005	0.19–0.54	0.02–0.08	9.4–47	24–158	2.5–30	29–136	IMST-152 (2005)
2007	0.10–0.65	0.02–0.06	3.1–31	32–170	3.2–39	27–132	IMST-180 (2008)
2008	0.22–0.54	0.02–0.09	12–37	46–148	4.6–35	30–135	IMST-180 (2008)
Middle–Inner Bays							
1995	–	0.22–0.42	5–62	64–300	12–70	23–260	Atgin et al. (2000)
1996	0.65–1.69	–	–	–	–	–	Kontas (2006)
1997	0.19–0.99	0.14–0.82	50–103	142–281	27–79	96–311	Kucuksezgin et al. (2006)
1998	0.18–0.86	0.06–0.42	36–108	131–200	31–45	85–248	Kucuksezgin et al. (2006)
2000	0.36–1.3	0.12–0.38	47–113	122–183	23–84	94–313	Kucuksezgin et al. (2006)
2001	0.38–0.62	0.05–0.55	61–110	171–295	32–77	86–286	Kucuksezgin et al. (2006)
2002	0.12–0.51	0.02–0.36	44–80	116–316	24–105	112–412	Kucuksezgin et al. (2006)
2004	0.12–0.52	0.03–0.33	35–119	136–246	22–109	105–379	Kucuksezgin et al. (2004)
2005	0.36–0.97	0.03–0.38	38–89	138–202	26–86	118–360	IMST-152 (2005)
2007	0.14–0.78	0.03–0.57	22–99	137–189	30–72	127–260	IMST-180 (2008)
2008	0.22–0.67	0.01–0.48	9.8–68	125–154	29–70	108–240	IMST-180 (2008)
Background	0.08	0.20	12.5	100	55	70	Taylor (1972)

[a] Dry weight

cement factories, beverage manufacturing and bottling, tanneries), compared to the outer parts of the bay.

The concentrations of Hg ranged between 0.10 and 0.99 $\mu g\ g^{-1}$ in the Outer Bay, while the range at stations in the Middle and Inner Bays was 0.12–1.3 $\mu g\ g^{-1}$ during 1996–2008. High levels of Hg were also found in the Outer Bay from a mercury mining site in the NW part of the bay. The higher Hg concentrations found at sampling stations in the Outer Bay are due to wastes that flow in from the Gediz River. The Gediz River drains a basin that is 15,616 km^2 in area and has an annual discharge rate of 40–70 $m^3\ s^{-1}$ (EIE 1984). The banks of the Gediz River are densely populated and include extensive agricultural lands and numerous manufacturing, food, and chemical production facilities.

The highest concentration of Cd (0.82 $\mu g\ g^{-1}$ dry weight; dwt) was found in the inner part of the bay in 1997. The concentrations of Cd measured in sediments varied; levels ranged from 0.009 to 0.33 $\mu g\ g^{-1}$ in the Outer Bay and 0.01 to 0.82 $\mu g\ g^{-1}$ in the Middle–Inner Bays.

Lead levels were quite high in the sediments of the middle and inner parts of the bay during 1998, 2000, and 2004. The main source of Pb probably derives from motorized traffic, because the great majority of vehicles have no catalytic converters and burn leaded fuel. The concentrations of Pb ranged between 9.8 and 119 and 3.1 and 94 $\mu g\ g^{-1}$ in the middle–inner and outer parts of the bay, respectively. The concentrations detected of Cr ranged between 19 and 199 $\mu g\ g^{-1}$ dwt in the Outer Bay. The highest Cr values were observed in the middle and inner parts of the bay and ranged from 116 to 316 $\mu g\ g^{-1}$ (Table 4). Until 1994, the leather tanning plants, which use large quantities of Cr in their tanning processes, discharged wastes directly into the Inner Bay. Maximum levels of Cr were observed in the estuary of the Gediz River.

The concentrations found of Cu ranged between 2.2 and 39 and 22 and 109 $\mu g\ g^{-1}$ in the Outer and Middle–Inner Bays, respectively. The concentrations of Zn ranged between 14 and 167 $\mu g\ g^{-1}$ in the Outer Bay, while the range at stations in the Middle and Inner Bays was 85–412 $\mu g\ g^{-1}$ during 1995–2008. The highest mean Cu and Zn values were measured during 2001, in the middle–inner parts of the bay.

Average crustal abundance values of the trace metals are often used as elemental background concentrations for comparison. Taylor's (1972) crustal abundance values were used in this review as background reference values. The Hg, Pb, Cr, and Zn concentrations that were measured in sediment samples exceeded the background levels at all stations (Kontas 2006; Kucuksezgin et al. 2004, 2006; IMST-152 2005; IMST-180 2008). Cd and Cu values were below the background values in the Outer Bay, while they were higher than background levels in the Middle–Inner Bays. Increasing heavy metal concentrations tend to be associated with fine-grained sediments and this can be seen in the high metal levels found in zones with clayey silt and silt. Similar results have been reported by Duman et al. (2004) in Izmir Bay.

The concentrations of trace metals in the bay were generally higher than the background levels from the Mediterranean and Aegean Seas, except that Cd and the Pb levels gradually decreased over the sampling period (MAP 1987; UNEP 1978;

Whitehead et al. 1985; Friligos et al. 1998; Batki et al. 1999). The levels of metals found are lower in the Inner Bay than in polluted areas of the Mediterranean Sea (Zvonaric and Odzak 1998; Ujevic et al. 1999).

Linear regression analysis was also used to analyze the data set of trace metal concentrations in the sediment. Table 5 displays all significant resultant values for Izmir Bay. A significant negative trend in time was observed in Pb concentrations. The decrease of Pb can mainly be attributed to the reduced lead discharge from the industrial activities. A slight decrease of Cd in the sediment from Izmir Bay was also noted.

Table 5 Results from the regression analyses of trace metals in sediment and fish from Izmir Bay: slope of the regression (standard error), coefficient, p level, and sample size (n)

Data	n	Slope of regression	Coefficient	p level
Sediment				
Hg	150	−0.009 (0.005)	0.023	n.s.
Cd	150	−0.062 (0.021)	0.056	<0.01
Pb	150	−3.48 (0.46)	0.28	<0.001
Cr	150	−0.85 (1.16)	0.004	n.s.
Cu	150	−0.11 (0.44)	0.0004	n.s.
Zn	150	0.65 (1.51)	0.001	n.s.
M. barbatus				
Hg	110	−0.069 (0.028)	0.052	<0.02
Cd	112	0.069 (0.068)	0.009	n.s.
Pb	106	−1.701 (2.51)	0.004	n.s.
Cr	42	0.58 (2.43)	0.001	n.s.
Cu	101	11.9 (3.02)	0.136	<0.001
Zn	99	38.1 (10.07)	0.129	<0.001

Mullus barbatus, being bottom dwellers to a certain extent and being mobile, are species that tend to concentrate contaminants to a higher degree than do other species. For this reason, it was recommended by FAO/UNEP (1993) that this organism should serve as a biomonitoring species. The concentrations of heavy metals found in *M. barbatus* have varied, with Hg ranging from 14 to 520, Cd from 0.10 to 10, Pb from 2.6 to 478, Cr from 22 to 270, Cu from 178 to 568, and Zn from 2157 to 3772 μg kg^{-1} wet wt in the bay. The descending order of heavy metal concentrations found in *M. barbatus* was Zn>Cu>Pb>Cr>Hg>Cd.

The highest metal levels were found as Hg 212, Cd 3.5, and Pb 491 μg kg^{-1} in *Solea vulgaris* in 1998, 2002, and 2001, respectively (Table 6). Hg and Cd levels were significantly lower in *S. vulgaris* than *M. barbatus*. The linear regression analyses performed on the overall data from 1996 to 2008 indicated highly positive trends in time for Cu and Zn concentrations in *M. barbatus*. However, a significant negative trend was established for Hg concentrations in this species (Table 5).

According to MAFF (1995), the metal concentrations in muscle of *M. barbatus* and *S. vulgaris* appear to be low compared with the limit value for human consumption of metals, i.e., 500 μg kg^{-1} for Hg, 200 μg kg^{-1} for Cd, 2000 μg kg^{-1} for Pb, 100 μg kg^{-1} for Cr, 20,000 μg kg^{-1} for Cu, and 50,000 μg kg^{-1} for Zn.

Table 6 Minimum and maximum concentrations of heavy metal levels found in fish taken from Izmir Bay ($\mu g\ kg^{-1}$ wet wt)

Period	Organism	Hg	Cd	Pb	Cr	Cu	Zn	References
1996	*M. barbatus*	182–259	0.13–2.4	2.6–125	–	–	–	Kucuksezgin et al. (2006)
	S. vulgaris	17–159	0.44–2.2	46–388	–	–	–	Kucuksezgin et al. (2006)
1997	*M. barbatus*	66–399	0.53–5.7	29–349	–	–	–	Kucuksezgin et al. (2006)
	S. vulgaris	5.2–95	1.1–2.3	110–364	–	–	–	Kucuksezgin et al. (2006)
1998	*M. barbatus*	27–285	0.77–1.6	8.0–478	22–199	178–530	2157–3727	Kucuksezgin et al. (2006)
	S. vulgaris	11–212	1.2–3.5	2.0–341	24–312	209–273	3394–6971	Kucuksezgin et al. (2006)
2000	*M. barbatus*	14–355	0.38–9.4	16–241	–	187–421	2522–3643	Kucuksezgin et al. (2006)
2001	*M. barbatus*	18–276	1.1–10	36–303	62–64	208–406	2436–3348	Kucuksezgin et al. (2006)
	S. vulgaris	4.5–91	1.8–2.1	90–491	–	121–290	3272–4445	Kucuksezgin et al. (2006)
2002	*M. barbatus*	34–520	0.10–10	17–300	–	367–568	3106–3771	Kucuksezgin et al. (2006)
	S. vulgaris	10–113	0.10–3.5	1.0–218	–	273–292	4724–5836	Kucuksezgin et al. (2006)
2003	*M. barbatus*	101–209	0.83–2.2	15–103	–	399–428	2926–3508	Kucuksezgin et al. (2006)
2004	*M. barbatus*	35–200	0.14–6.6	8.1–59	23–270	262–539	3032–3772	IMST-152 (2004)
2005	*M. barbatus*	64–166	2.0–7.9	40–236	29–184	267–544	2748–3742	Gonul and Kucuksezgin (2007)
2007	*M. barbatus*	97–246	0.67–2.3	143–212	37–90	426–451	3272–3664	IMST-167 (2007)
2008	*M. barbatus*	149–184	0.93–1.1	40–44	84–110	375–524	3287–3500	Kucuksezgin (2009)

Data on the heavy metal concentrations found in this review exceeded those found in clean areas of the Aegean and Mediterranean Seas (IMST 1995, 1997; Kucuksezgin et al. 2001), and these likely resulted from natural and industrial inputs to Izmir Bay. Conversely, the metal concentrations in fish from Izmir Bay are considerably lower than those found in the polluted areas of Mediterranean Sea.

Concentrations of trace metals were measured along the coasts of Izmir Bay in the mussel (*Mytilus galloprovincialis*) from 2004 to 2008 (Table 7). Kucuksezgin et al. (2008) reported higher heavy metal levels for this organism in the inner part of the bay. The highest levels of Hg and Cd were observed in the Outer Bay (non-industrial area) and resulted from past mercury mining activities (IMST-165 2006, 2007, 2008). Maximum concentrations of Hg were also measured in sediments from the same location (Kucuksezgin et al. 2006; Kontas 2006). Pb, Cu, and Zn levels were higher in the Inner Bay than in the outer and middle parts of the bay. Sunlu (2006) found higher concentrations of Cd and Pb in different parts of the bay, and the author attributed the differences to using a different analysis method (flame atomic absorption spectrometry; AAS). The maximum trace metal concentrations ($\mu g\ g^{-1}$ dwt) for mollusks were compared with the Spanish legislative limits for these pollutants, i.e., 1 $\mu g\ g^{-1}$ for Cd, 5 $\mu g\ g^{-1}$ for Pb, 20 $\mu g\ g^{-1}$ for Cu, and no allowable detection for Cr (Torres et al. 2002). Metal content in mussels collected at Izmir Bay appears to be low for Cu and Cr but high for Cd and Pb, when compared with these Spanish legislative limits. Kucuksezgin et al. (2010) studied heavy metal concentrations and levels of fecal pollution in specimens of the clam (*Tapes decussatus*) collected from Izmir Bay. Levels in this organism were generally found to increase during the warmer periods of the year, especially for Cu, Zn, and Cr, all of which are

Table 7 Trace metal concentrations detected in *M. galloprovincialis* taken from two sampling sites in Izmir Bay from 2004 to 2008

Period	Metals	Inner Bay (min–max)	Middle Bay (min–max)	Outer Bay (min–max)	References
2004	Hg	0.018–0.018	0.017–0.018		Kucuksezgin et al. (2008) ($\mu g\ g^{-1}$ dwt)
	Pb	0.38–0.42	0.068–0.078		
	Cr	0.171–0.173	0.114–0.118		
	Cu	5.26–5.30	3.56–3.78		
	Zn	26.9–28.5	17.9–18.0		
2002–2003	Cd	0.48–0.52	0.45–0.50	0.28–0.32	Sunlu (2006) ($\mu g\ g^{-1}$ wet wt)
	Pb	1.67–1.72	0.80–1.05	1.39–1.45	
	Cu	1.79–1.85	1.43–1.55	1.58–1.65	
	Zn	34.3–37.2	20.0–21.9	22.6–23.0	
2006–2008	Hg	0.050–0.081	0.052–0.13	0.10–0.18	IMST-165 (2006, 2007, 2008) ($\mu g\ g^{-1}$ dwt)
	Cd	0.69–0.97	0.74–2.01	0.94–5.27	
	Pb	2.72–5.97	1.43–2.84	0.91–4.07	
	Cr	1.99–3.66	2.43–8.58	2.74–5.48	
	Cu	7.40–11.1	7.37–9.55	6.30–8.46	
	Zn	116–141	97–133	105–144	

essential for life. The natural seasonal variation in metal content can depend on several factors that are not easily differentiated. The levels of metal contaminants found in *T. decussatus* in Izmir Bay were lower than those of *M. galloprovincialis* collected in the same area. Trace metal (Cu, Cr, Zn, Ni) concentrations in *T. decussatus* at the sampling site were higher than those in *M. galloprovincialis* in the Thermaikos Gulf, Greece (Catsiki and Florou 2006), and Hg, Cd, Pb, Zn levels were similar to those in southern Spain in *Chamelea gallina* (Usero et al. 2005)

3.3 Organochlorine Pesticides and PCBs

OCPs have been heavily used in the Mediterranean Region, but their production and usage is banned, with some exceptions, in the majority of the countries of the region as a consequence of the application of the PIC (Prior Informed Consent) protocols. Aldrin, dieldrin, heptachlor, chlordane, and the benzene hexachlorides (BHCs) are prohibited in the EU for use in plant protection, whereas for other applications a written authorization for import is needed (UNEP 2002; Senthil Kumar et al. 2005). The variation of OCP residue profiles clearly showed that OCPs were widely used between the 1950s and 1980s in Turkey. Turkey signed the document entitled "Protocol for the protection of the Mediterranean Sea against pollution from land-based sources and activities" in 1988.

In addition to receiving nutrient-enriched effluents, the Gediz River also comprises waters that have high concentrations of pesticides. Filiz and Kucuksezgin (2008) studied pesticide levels in the Gediz River surface sediments from January to July 2004. These authors found an average ΣOCP level of 15.2 ng g^{-1} dwt (range 1.4–49.2 ng g^{-1} dwt) in the rainy season. In the dry season, these values were in the range of 5.4–55.7 ng g^{-1} dwt, with an average value of 24.0 ng g^{-1} dwt. Concentrations of ΣOCP, PCBs, and Σ16 PAHs in *M. galloprovincialis* (ng g^{-1} dwt) are given in Table 8 from three sites in Izmir Bay that were sampled from 2006 to 2008.

Table 8 Concentrations of organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), and polyaromatic hydrocarbons (PAHs) detected in *M. galloprovincialis* (ng g^{-1} dwt) taken from Izmir Bay during 2006–2008

Period	Region	OCPs	PCBs	PAHs	References
2006	Outer	5.0–5.1	9.8–12.1	532–662	IMST-165 (2006)
	Middle	11.0–11.2	48.6–49.9	1950–1987	
	Inner	34.7–38.6	79.8–87.5	4238–4314	
2007	Outer	6.8–7.8	bdl[a]	400–428	IMST-165 (2007)
	Middle	10.4–11.4	15.6–17.0	713–722	
	Inner	28.4–31.4	52.3–52.5	1852–1874	
2008	Outer	10.3–11.0	9.2–10.3	282–291	IMST-165 (2008)
	Middle	6.7–7.0	12.3–14.6	650–663	
	Inner	0.26–0.35	40.6–46.7	1726–1729	

[a] Below detection limit

3.4 *Aliphatic and Aromatic Petroleum Hydrocarbons in Sediment*

In terms of the Σ5 aliphatic and Σ16 aromatic hydrocarbons (Table 9), the concentrations detected in sediments of Izmir Bay ranged from 84 to 4427 and 2.5 to 113 ng g^{-1} dwt (Darilmaz and Kucuksezgin 2007). Each sample was quantified for its content of the following: Σ5 aliphatics – *n*-tetradecane, *n*-heptadecane, pristane, *n*-octadecane, and phytane; Σ16 PAHs of concern to USEPA – the sum of 6 low molecular weight aromatics (i.e., naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene) and 10 high molecular weight aromatic rings (i.e., fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno [1,2,3-c,d]pyrene, dibenzo[a,h]anthracene, benzo[g,h,i]perylene). The highest levels of the Σ5 aliphatic hydrocarbons were found in the Inner Bay. Such high concentrations are probably linked to anthropogenic sources. Sampling stations from the Inner Bay are subject to more restricted water circulation, where eutrophication is common and results in the development of blooms from the availability of excessive nutrient and organic matter (Kucuksezgin et al. 2006). The concentrations of Σ5 aliphatics found in sediment samples from the Izmir Bay were generally less than 1 μg g^{-1} dwt, except for samples collected from the Inner Bay stations.

Table 9 Concentrations of Σ16 PAHs (ng g^{-1} dwt), extractable organic matter (EOM, mg g^{-1}), and organic carbon (mg C g^{-1}) in Izmir Bay sediments during 2000 (Darilmaz and Kucuksezgin 2007)

Region	Σ5 Aliphatics	Σ16 PAHs	EOM	Organic carbon
Outer Bay	84–473	2.5–53	0.99–7.10	8.6–27.0
Middle Bay	303	28	11	23.0
Inner Bay	1279–4427	38–113	1.7–84	33.1–72.5

Aliphatic hydrocarbon concentrations in the Inner Bay were higher than those in the Crete, eastern Mediterranean area (Gogou et al. 2000), and similar to those analyzed in the Black Sea and Bosphorus (Readman et al. 2002). The PAH levels found in samples reviewed in the present study were consistent with those reported for open sea sediments of the Aegean Sea, Kavala Bay, Black Sea coastline, and northwestern Black Sea (Maldonado et al. 1999; Gogou et al. 2000; Hatzianestis and Sklivagou 2001; Readman et al. 2002; Papadopoulou and Samara 2002).

4 Conclusions

From this review the following conclusions are drawn:

1. The aquatic environment of the Izmir Coastal System is characterized, in general, by having high nutrient concentrations throughout the year. Nutrients are present

at levels well above the background values from the open Aegean Sea, especially in the Inner and Middle Bays.

2. Linear regression analyses of nutrient data showed significant negative trends in the Outer Bay for phosphate and nitrate, while a negative trend was observed for nitrate levels in the Middle–Inner Bays. Positive trends were found for silicate that existed in different parts of Izmir Bay.
3. Pollution in the Outer Bay is insignificant; however, there is high eutrophication in the Inner Bay and it may be spreading progressively to the outer part of the bay. In the beginning of 2000, a Wastewater Treatment Plant began operating to treat wastes entering the bay. Even though the capacity of this plant is sufficient for removing nitrogen from the wastes, it is inadequate for removing phosphate. This is also illustrated by the results of the linear regression analysis performed on the nutrient data. The quality of the marine environment in the middle and inner parts of the bay has not yet noticeably improved. Nutrient concentrations remain relatively high in the surface layers of the Gediz River estuary.
4. The concentrations of heavy metals in Bay sediment were generally higher than similar background levels from the Mediterranean and Aegean Seas. The Gediz River is the major source of anthropogenic input into the Outer Bay due to insufficient treatment of wastes. If all monitoring data from the surficial sediment of Bay are used, no significant trend exists other than a decrease in Pb and slight decrease in Cd. High levels of total petroleum hydrocarbon residues were found in the Inner Bay from anthropogenic activities; these derived mainly from combustion processes resulting from marine traffic and industrial activities.
5. Average Hg and Cd concentrations in fish were found to be higher than those reported from the unpolluted areas of the Aegean and Mediterranean Seas. A negative trend in time was detected for Hg, while positive trends were observed for Cu and Zn. Future monitoring will demonstrate whether the detected trends persist.
6. Future research in Izmir Bay should include the study of sensitive biomarkers of heavy metals and PAHs in fish and mussel tissue. Increases in industrialization and population on the banks of the Gediz River are expected to continue, hence further monitoring of its output into the bay is essential.
7. The most frequently detected trace metals were Hg, Cd, Pb, Cr, Cu, and Zn, and the most prominent organic pollutants found were the organochlorine pesticides and petroleum hydrocarbons.
8. The burden of accumulated pollution that has drained into the bay from surrounding regions during the last 5–6 decades has affected the quality of the Izmir Bay ecosystem.

5 Summary

About 30 studies from the published literature were reviewed to determine the status of several categories of pollutants (i.e., nutrients, heavy metals, and organic compounds) in the Izmir Bay. The Izmir Bay of western Turkey is one of the great natural bays of the Mediterranean Sea. The city of Izmir is an important industrial and commercial center and is a cultural focal point for this area. In this review, I review the results of studies performed by researchers over a period of two decades to glean what probable impact has resulted from human activities in the bay. I also summarize available data on the contamination levels found in the bay and the effects generated by these levels on the ecosystem of the bay. In addition, I provide a monitoring analysis that provides the distribution of inorganic nutrients and chlorophyll-a that have been investigated and the N/P ratios that were determined in Izmir Bay during 1996–2008. To identify the temporal trends in the monitoring data, linear regression analyses were performed. Significant negative trends were observed in TNO_x concentrations in Izmir Bay. No significant trend was found in metal concentrations except Pb in the sediment. A significant negative trend existed for Hg in fish. Unfortunately, there are only limited data available for the organic contaminants from Izmir Bay. Therefore, any attempt to create a regional scale assessment from organic contaminant data available in the literature is limited by the scarcity of available information.

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Teratogenicity and Embryotoxicity in Aquatic Organisms After Pesticide Exposure and the Role of Oxidative Stress

Veronika Pašková, Klára Hilscherová, and Luděk Bláha

Contents

1 Introduction

Complex factors have contributed to the decline of aquatic populations worldwide. Among these factors are intensification of agriculture, including the application of fertilizers and agents of crop protection, and loss of habitat. Various developmental abnormalities in natural populations of aquatic vertebrates have been documented, and agricultural pesticides are considered by many to be one of the important factors that cause such abnormalities. Amphibians may potentially be a target of environmental stressors and toxicants as a result of their biphasic life cycles and skin permeability. In this chapter, the role of oxidative stress in the teratogenic action of pesticides is reviewed and addressed, with special attention given to non-target aquatic organisms such as amphibians, fish, and invertebrates. The review of

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available literature indicates that many pesticides enhance oxidative stress in aquatic organisms, and such stress may be linked to developmental alterations, including reproductive effects, embryotoxicity, and/or teratogenicity.

Any external factor affecting cellular proliferation, differentiation, or apoptosis can produce embryotoxic or teratogenic effects, and such factors include chemical exposures at high concentrations; such effects may result in permanent congenital malformations, functional abnormalities, or even embryo death (Gilbert 2006). Several external factors may result in embryotoxicity and teratogenicity in the aquatic environment. These factors include ultraviolet radiation, extremes in pH, thermal and ionic conditions, infections, parasites, as well as chemicals such as pharmaceuticals, retinoid and aromatic compounds, and pesticides (Ankley et al. 2004; Bilski et al. 2003; Blaustein and Johnson 2003; Finn 2007; Hayes et al. 2006).

One mechanism by which chemicals induce toxicity is through oxidative stress, and it has been shown that several widely used pesticides are capable of producing pro-oxidants in cells (Tellez-Bańuelos et al. 2009; Vismara et al. 2001a). Furthermore, oxidative stress is the major mechanism by which some pesticides exert their effects; a prime example is the bipyridyl herbicides (Ruiz-Leal and George 2004; Sewalk et al. 2000).

Pesticides are regularly applied onto agricultural land worldwide, and the resultant timing of exposure often parallels the appearance of the early developmental stages of aquatic organisms (Greulich and Pflugmacher 2003). Although the various side effects that pesticides have on biota have been documented in many studies, to our knowledge, no consistent overview of pesticide embryotoxicity in aquatic invertebrates and vertebrates is available. Hence, in this review, we summarize the existing knowledge on this topic. Moreover, we present an overview of available information on the general teratogenic and embryotoxic effects of pesticides in aquatic biota such as fish, amphibia, and invertebrates. In this review we emphasize toxic effects that are related to oxidative stress and draw lines of evidence to support the view that it is a possible toxicity mechanism behind the induction of teratogenicity.

2 Pesticides and Teratogenicity in Fish

In fish, developmental malformations have been linked to the presence of several environmental pollutants such as persistent organochlorines, pesticides, or heavy metals (Westernhagen von 1988). In several studies, direct embryotoxicity has resulted from the presence of complex matrices such as oil (Heintz et al. 1999), and recently, tests for embryonic malformations in fish have been used as general water quality indicators (Klumpp et al. 2002).

The array of effects that pesticides have had on embryonic development in fish is summarized in Table 1.

Zebra fish (*Danio rerio*; family Cyprinidae) constitutes the most common model of test fish species, and it has been used in many pesticide studies; the results from Strmac and Braunbeck (1999) and Osterauer and Köhler (2008) are examples. Other species used in pesticide testing schemes include the Japanese medaka (*Oryzias latipes*; family Adrianichthyidae; Villalobos et al. 2000) and various salmonids

Table 1 The effects of selected pesticides on development and reproduction in fish

Pesticide	Organism	Toxic effect	References
Carbamates			
Thiobencarb	*O. latipes*	Bradycardia, pericardial edema, hemostasis, poor yolk resorption, cephalic and spinal deformities, abnormal hatching	Villalobos et al. (2000)
Dithiocarbamate	*D. rerio*	Twisted notochord, reduced hatching notochord distortions	Haendel et al. (2004), Tilton et al. (2006, 2008)
		Distorted notochord development and shortened anterior to posterior axis	van Boxtel et al. (2010)
Thiuram	*D. rerio*	Wavy notochords, disorganized somites, shortened yolk sac extension	Teraoka et al. (2006)
Carbaryl	*D. rerio*	Red blood cell accumulation, delayed hatching and pericardial edema, bradycardia	Lin et al. (2007)
Synthetic pyrethroids			
Esfenvalerate	*O. latipes*	Deleterious reproductive effects, reduced hatching success, and larval viability	Werner et al. (2002)
	Oncorhynchus tshawytscha	Myoskeletal abnormality, lordosis	Viant et al. (2006b)
Deltamethrin	*Cyprinus carpio*	Deleterious reproductive effects, decreased hatching success, larvae lethality, altered development	Köprücü and Aydin (2004)
	O. mykiss	Fry lethality, loss of equilibrium	Ural and Saglam (2005),
	Brachydanio rerio	enhanced larvae mortality, reduced hatching rate	
	D. rerio	Embryolethality, neurobehavioral effects – spastic movements, pericardial edema, craniofacial abnormalities	Görge and Nagel (1990), and DeMicco et al. (2010)
Cypermethrin	*C. carpio*	Embryolethality, reduced hatching success	Aydin et al. (2005)
	D. rerio	Embryolethality, neurobehavioral effects – spastic movements, pericardial edema	DeMicco et al. (2010)
Bifenthrin	*D. rerio*	Embryolethality, curvature of the body axis, neurobehavioral effects – spastic movements	DeMicco et al. (2010)
Λ-Cyhalothrin	*D. rerio*	Embryolethality, neurobehavioral effects – spastic movements, pericardial edema	DeMicco et al. (2010)

Table 1 (continued)

Pesticide	Organism	Toxic effect	References
Resmethrin	*D. rerio*	Neurobehavioral effects – spastic movements	DeMicco et al. (2010)
Permethrin	*O. latipes*	Delayed swim bladder inflation, inability of hatchling to respond to stimulus; uncoordinated movements, myoskeletal defects, and transient enlargement of gall bladder of larvae	González-Doncel et al. (2003)
	D. rerio	Embryolethality, curvature of the body axis, neurobehavioral effects – spastic movements, craniofacial abnormalities	DeMicco et al. (2010)
Organophosphates			
Malathion	*D. rerio*	Reduced growth, survival, and eye diameters	Cook et al. (2005)
	Clarias gariepinus	Deformed notochord and pericardial edema, larvae with bent body, and swollen yolk sac	Lien et al. (1997)
	Sciaenops ocellatus	Decreases in growth in weight of larvae, increased protein synthesis	McCarthy and Fuiman (2008)
	Melanotaenia fluviatilis	Moderate larval lethality	Reid et al. (1995)
Diazinon	*C. carpio*	Deleterious reproductive effects, reduced hatching success, and larval viability	Aydin and Köprücü (2005)
	O. latipes	Edemas both along vitelline veins and within the pericardial sac, delay in hatching, decrease in swim bladder inflation, decreased length	Hamm and Hinton (2000)
	D. rerio	Embryolethality, decreased heart rate, yolk sac and heart sac edema, spine deformations, altered hatching date	Osterauer and Köhler (2008)
Chlorpyrifos	*D. rerio*	Increased locomotor activity, paused jerky movements, heart edema, spinal deformity	Kienle et al. (2009)

Table 1 (continued)

Pesticide	Organism	Toxic effect	References
Organochlorines			
Endosulfan	*O. latipes*	Delayed hatching, smaller fry, altered mobility	Gormley and Teather (2003)
	D. rerio	Mild trunk curvature, abnormal behavior, edema, microcephaly, and impaired movement, along with increased death rate	Willey and Krone (2001)
Lindane	*S. aurata*	Myoskeletal defects, skin opacity, exophthalmia, weak swimming, depigmentation, behavioral changes	Oliva et al. (2008)
	O. mykiss	Hepatocytic alterations (glycogenic depletion, RER and dictyosome changes, secondary lysosome accumulation)	Sylvie et al. (1996)
	B. rerio	Enhanced larvae mortality, decreased growth	Görge and Nagel (1990)
Thiacloprid	*D. rerio*	Heart rate affection	Osterauer and Köhler (2008)
Organosulfurs			
Methyl isothiocyanate	*D. rerio*	Notochord distortions	Tilton et al. (2006)
Terbutryn (a triazine) + triasulfuron	*S. aurata*	Curvatures of the vertebral column, the hepatocytes forming slackly arranged cords, loss of cellular shape of hepatocytes, lipid inclusions, nuclear pyknosis	Arufe et al. (2004b)
Phenylpyrazole			
Fipronil	*D. rerio*	Notochord degeneration, shortening along the rostral–caudal body axis, ineffective tail flips	Stehr et al. (2006)
Triazines			
Atrazine	*S. ocellatus*	Altered growth, hyperactivity, and faster active swimming speed (elevated rate of energy utilization) Declines in growth in wet weight and protein content in larvae, increase in rates of protein degradation	del Carmen Alvarez and Fuiman (2005), McCarthy and Fuiman (2008)
	B. rerio	Enhanced larvae mortality, increased number of deformations and edema	Görge and Nagel (1990)
Simazine	*S. aurata*	Reduced larvae survival, hepatic lesions, loss of cellular shape in hepatocytes, lipid inclusions, focal necrosis, abundant nuclear pyknosis	Arufe et al. (2004a)

Table 1 (continued)

Pesticide	Organism	Toxic effect	References
Cyanazine	*M. fluviatilis*	Moderate mortality with decreasing trend from the day of hatching	Reid et al. (1995)
Organotins			
Triphenyltin acetate	*D. rerio*	Larval mortality, delayed hatching, skeletal malformation, retarded yolk sac resorption, and edema in the heart and yolk sac regions, histo- and cyto-pathological alterations of larval liver including changes in nuclei and mitochondria as well as glycogen depletion	Strmac and Braunbeck (1999)
Dinitrophenol			
Dinoseb	*O. latipes*	Larval mortality, reduced eye growth, diminished heart rate, failed hatching, developmental retardation, pericardial edema, and reduced growth, reductions of eye area and width	Viant et al. (2006a, b)

RER, rough endoplasmic reticulum

such as *Oncorhynchus* sp. (family Salmonidae; Sylvie et al. 1996). The testing that has utilized these species has involved a wide array of pesticides and pesticide classes. For example, the toxicity of the following classes has been tested in these fish species: organophosphates, triazines, synthetic pyrethroids, carbamates, organochlorines, some studies with organosulfur pesticides (Arufe et al. 2004b; Tilton et al. 2006), phenylpyrazoles (Stehr et al. 2006), organotins (Strmac and Braunbeck 1999), and dinitrophenols (Viant et al. 2006a, b).

The array of pesticide effects that have been observed on fish embryonic development has included malformations in myoskeletal development (such as notochord abnormalities of degeneration), defects along the rostral–caudal body axis, curvature of the vertebral column, and reduced growth (McCarthy and Fuiman 2008; DeMicco et al. 2010; van Boxtel et al. 2010). In other studies, pesticides affected various visceral organs in ways that led to defects in the hepatic, cephalic, and eye region, and various edemas in pericardial area or yolk sac (Strmac and Braunbeck 1999; Hamm and Hinton 2000; Willey and Krone 2001). Besides these morphological alterations, embryonic and larval exposures to pesticides have also resulted in decreased hatching success and larval mortality (Görge and Nagel 1990; Aydin and Köprücü 2005; Viant et al. 2006a, b) or behavioral alterations such as uncoordinated movements and loss of balance (Ural and Saglam 2005; González-Doncel et al. 2003; Kienle et al. 2009).

As is clearly shown in Table 1, various pesticides have produced significant detrimental effects on developmental processes in different fish species. We address these effects and the role of oxidative stress in developmental toxicity in more detail below (Sections 5 and 6).

3 Pesticides and Teratogenicity in Amphibians

Amphibians are known to be highly sensitive organisms and can be affected by chemical, physical, and habitat factors; moreover, it is believed that pesticides are one of the causes of the worldwide decline in amphibian populations (Muths et al. 2006). Most amphibian species breed during the spring when pesticides are being applied onto the land for weed, fungal, insect, or other pest control, which makes amphibians highly vulnerable to pesticide toxicity (Greulich and Pflugmacher 2003).

In natural frog populations, morphologically malformed individuals usually constitute a small fraction of less than 2% (Ouellet 2000). However, a much higher incidence (up to 60%) of malformed specimens was documented to occur in contaminated ponds (Meteyer 2000). In agricultural ecosystems, developmental malformations resulting from pesticide exposure were documented to have occurred in several amphibian species in India (Gurushankara et al. 2007) or in Canada (Ouellet et al. 1997).

Embryotoxicity and teratogenicity of various pesticide classes have been documented to have occurred in amphibians in laboratory studies and in field observation studies (Table 2). Most of the studies used the prototypical model organism *Xenopus laevis* from the Pipidae family, but species from other families that included Ranidae, Bufonidae, Microhylidae, and others were also used.

Table 2 The effects of selected pesticides on development and reproduction in amphibians

Pesticide	Organism	Toxic effect	References
Mixture of pesticides	*Rana pipiens*	Altered development and growth, sex differentiation, behavior, timing of initiation, and completion of metamorphosis	Hayes et al. (2006)
Carbamates			
Carbofuran	*Microhyla ornata*	Blistering, distention of body cavities, curvature of the body axis, poor blood circulation, retarded growth, abnormal behavior, poor pigmentation	Pawar and Katdare (1984)
Carbamate ZZ-Aphox	*Rana perezi*	Histological damage of gill, liver, gall bladder, heart, and notochord	Honrubia et al. (1993)
Carbaryl	*Ambystoma barbouri*	Delayed hatching, reduced larval survival, lower growth rates, respiratory distress, limb deformities	Rohr et al. (2003)
	X. laevis	Abnormal tail flexure, skeletal muscle lesions, wavy or bent notochord	Bacchetta et al. (2008)
	Rana sphenocephala	Increased length of tadpoles, larger mass of metamorphosis	Bridges and Boone (2003)
Organophosphates			
Fenitrothion	*M. ornata*	Blistering, distention of body cavities, curvature of the body axis, poor blood circulation, retarded growth, abnormal behavior, poor pigmentation	Pawar and Katdare (1984)
	R. pipiens, Rana clamitans, Rana catesbeiana	Embryotoxicity, behavior alterations, paralysis	Berrill et al. (1994)
Guthion and guthion 2S	*X. laevis*	Embryolethality, decreased body length, developmental alterations	Schuytema et al. (1994)
Malathion	*X. laevis*	Defects of neuromuscular activity such as spasms, tremors, and affected swimming, abnormal tail flexure, distorted myocytes	Bonfanti et al. (2004)
	Ambystoma mexicanum	Embryonic mortality, delayed or stopped development, not completed neurulation, thin notochord, not fused neural folds, embryos without notochord and neural canal and located edema, erratic swimming	Robles-Mendoza et al. (2009)

Table 2 (continued)

Pesticide	Organism	Toxic effect	References
Chlorpyrifos	*X. laevis* *Hyla chrysoscelis* *Rana sphenocephala,* *Acris crepitans*	Reduced myotome size and hypertrophies; defects of neuromuscular activity such as spasms, tremors, and affected swimming, notochord flexure, distorted myocytes tadpole mortality, swim speed affection, lower mass of tadpoles	Colombo et al. (2005) Bonfanti et al. (2004) Widder and Bidwell (2008)
	Gastrophryne olivacea *Bufo bufo gargarizans* *A. mexicanum* *Rana boylii*	Shrunken fins, tail deformities, and head edema, behavioral changes, micronucleus induction, tadpole lethality thin notochord and neural canal, lateral tail flexure, convulsions, spasms and tremors, larval mortality	Yin et al. (2009) Robles-Mendoza et al. (2009) Sparling and Fellers (2007)
Diazinon	*Bufo melanostictus* *Polypedates cruciger*	Larval mortality, altered activity, growth retardation	Sumanadasa et al. (2008)
	R. boylii	Larval mortality	Sparling and Fellers (2007)
Organochlorines			
Dieldrin	*Limnodynastes tasmaniensis*	Abnormal otolith, otic capsule, and cephalic pigmentation	Brooks (1981)
Benzene hexachloride	*M. ornata*	Blistering, distention of body cavities, curvature of the body axis, poor blood circulation, retarded growth, abnormal behavior, poor pigmentation	Pawar and Katdare (1984)
Methoxychlor	*X. laevis*	Altered hind limb differentiation and tail resorption, interference with normal reproductive processes	Fort et al. (2004a, b)
	Xenopus tropicana	Altered rate of larval development, delayed development, chronic reproductive effects	
	Ambystoma macrodactylum	Altered startle responses and a shorter distance of travel following application of the startle stimulus, increased predation, mortality of larvae	Eroschenko et al. (2002)
Endosulfan	*Litoria citropa*	Reduced tadpole survival, increased vulnerability to predation	Broomhall (2002)

Table 2 (continued)

Pesticide	Organism	Toxic effect	References
Endosulfan and octylphenol	*A. barbouri*	Delayed hatching, reduced larval survival, lower growth rates, respiratory distress, limb deformities	Rohr et al. (2003)
Heptachlor	*Rana kl. Esculenta*	Decreased survival rate in tadpoles, alterations in the epidermis of tadpoles containing dilated and irregular vesicles, damaged mitochondria showing altered cristae	Fenoglio et al. (2009)
Pyridine			
Triclopyr	*R. pipiens*, *R. clamitans*, *R. catesbeiana*	Embryotoxicity, behavior alterations, paralysis	Berrill et al. (1994)
	R. pipiens	Reduced survival of tadpoles	Chen et al. (2008)
Bipyridyl			
Paraquat	*X. laevis*	Embryolethality, growth retardation, ventral tail flexure, abnormal somites; mitose alterations, necrotic myocytes, malformed intersomitic boundaries, altered swimming activity, embryolethality or embryos unable to swim, general reduction of length, medial flexures of the notochord and stunting	Vismara et al. (2000, 2001a, b), Mantecca et al. (2006), Osano et al. (2002)
Phenoxy carboxylic acid			
4-Chloro-2-methylphenoxyacetic acid	*X. laevis*	Growth retardation	Bernardini et al. (1996)
Chloroacetanilide			
Alachlor	*Bufo americanus* *R. pipiens*	Embryolethality, developmental alterations	Howe et al. (1998)

Table 2 (continued)

Pesticide	Organism	Toxic effect	References
Triazine			
Atrazine	*B. americanus*	Embryolethality, edemas	Howe et al. (1998)
	R. pipiens	Induced intersex animals	Carr et al. (2003)
	X. laevis	No effect on mortality, growth, time to metamorphosis, gonad and laryngeal development, or aromatase activity, sexual differentiation affection – increases in female ratios, no effects on reproduction, hatching success, or development	Coady et al. (2005), Oka et al. (2008), Du Preez et al. (2008)
	Rhinella arenarum	Embryolethality, greater mortality of larvae, alterations in the timing of metamorphosis	Brodeur et al. (2009)
Terpenoids			
Methoprene	*R. pipiens*	Severe developmental effects, dysmorphogenesis, high mortality	Ankley et al. (1998)
Methoprene degradation products	*X. laevis*	Eye, cranial, facial defects, spinal curvature and heart and gut malformations, dysmorphogenesis of craniofacial region, edemas, microphthalmia, reductions in the prosencephalon and mesencephalon, developmental delay	La Clair et al. (1998), and Degitz et al. (2003)
Amidine			
Amitraz	*X. laevis*	Growth retardation, edemas of the face, heart, and/or abdomen and axial flexures (curvature of the notochord or bending of the tail)	Osano et al. (2002)
2,4-Dimethylaniline	*X. laevis*	Subtoxic stimulation of growth, loss of pigment together with encephalomegaly, welling of the brain	Osano et al. (2002)

Similar to what occurs in fish, amphibians are known to be highly sensitive to several developmental effects; myoskeletal system, abnormal tail formation, and limb differentiation are among the most often reported effects caused by pesticide exposure (Fort et al. 2004a, b; Bacchetta et al. 2008). Further alterations include incomplete neurulation, edemas, epidermal defects, or gut malformations (Degitz et al. 2003; Robles-Mendoza et al. 2009), as well as severe dysmorphogenesis, embryonic and larval lethalities, delayed hatching, growth retardations, or altered metamorphosis (Vismara et al. 2000, 2001a, b; Brodeur et al. 2009).

In addition to investigations that have been performed with frogs and toads, a few studies were also performed with salamanders (family Ambystomatidae) exposed to pesticides; effects observed included larval mortality, limb deformities, and behavioral changes (Eroschenko et al. 2002; Robles-Mendoza et al. 2009; Rohr et al. 2003).

Similar to observations that have been made in fish studies (Table 1), testing results with amphibians (Table 2) indicate that significant embryotoxicity/teratogenicity and developmental toxicity result from pesticide exposure, and evidence suggests that oxidative stress may play a role in producing such effects.

4 Pesticides as Possible Teratogens in Invertebrates

Pesticides may not only alter development and reproduction in vertebrates but also affect various aquatic invertebrates. Data from studies that have documented developmental effects in invertebrates are presented in Table 3. Most invertebrate reproduction or developmental studies were performed with organophosphate and organochlorine insecticides (Key et al. 2007; Lee and Oshima 1998), although the effects of other pesticide classes (e.g., synthetic pyrethroids, chloroacetanilides and terpenoids; triazines, carbamates, azoles, and phenylpyrazoles) were also studied.

Among the effects found in gastropods, bivalve mollusks, echinoids, and decapod crustaceans were embryonic and larval lethality (Key et al. 2007; Harper et al. 2008), decreased hatching success or delayed hatching times (Lee and Oshima 1998; Sawasdee and Köhler 2009), as well as alterations in embryolarval development, and larval deformities (Bhide et al. 2006; Buznikov et al. 2007).

Model aquatic invertebrate organisms such as the cladoceran *Daphnia* were also investigated (Palma et al. 2009), but in this species, embryolethality was often masked by changes in other parameters, such as adult immobilization or number of offspring (Abe et al. 2001). Embryos and larvae of ascidian *Phallusia mammillata* were found to be a sensitive model, and the azole pesticides imazalil and triadimefon inhibited sperm viability and fertilization rate, and deregulated organogenesis of the nervous system in this species (Pennati et al. 2006).

Although oxidative stress possibly plays a role in the toxicity of pesticides to invertebrates as described in Table 3, to our knowledge no specific studies exist that link embryotoxicity directly to oxidative stress.

Table 3 The effects of selected pesticides on development and reproduction in aquatic invertebrates

Pesticide	Organism	Toxic effect	Reference
Carbamate			
Propoxur	*Lymnaea stagnalis*	Increased mortality, decreased hatchability, and larval deformities linked with deviation in protein fractions	Bhide et al. (2006)
Organophosphates			
Chlorpyrifos	*Strongylocentrotus droebachiensis*	Neurotoxicity, specific malformation – “mushroom”-shaped larva formation	Buznikov et al. (2007)
	M. galloprovincialis	Reduction of embryogenesis success	Beiras and Bellas (2008)
	Palaemonetes pugio	Lethality of newly hatched larvae and postlarvae	Key et al. (2007); Lee and Oshima (1998)
	Callinectes sapidus	Inhibited hatching	Palma et al. (2009)
	Daphnia magna	Underdeveloped second antennae, curved and incurved shell spine, arrested eggs, reduced number of offspring per female, embryos remaining at certain stage	
		Early larvae lethality, hatching time	
Dichlorvos	*L. stagnalis*	Increased mortality, decreased hatchability, and larval deformities	Bhide et al. (2006)
Diazinon	*Paracentrotus lividus*	Decreased length of primary mesenchymal branches and plutei, altered speed of development	Morale et al. (1998)
Malathion	*P. pugio*	Lethality of larvae, lower number of instars to post-larvae, lethality of newly hatched larvae	Key et al. (1998, 2007)
Azinphos methyl	*P. pugio*	Lethality of newly hatched larvae	Key et al. (2007)
Organochlorines			
Methoxychlor	*Strongylocentrotus purpuratus*	Disruption of gastrulation, abnormal cleavage, and gut development	Green et al. (1997)
Methoxychlor, dieldrin, lindane	*P. lividus*	Decreased rate of fertilization, increased polyspermy, mitotic alterations, altered development, intracellular Ca homeostasis	Pesando et al. (2004)

Table 3 (continued)

Pesticide	Organism	Toxic effect	Reference
Lindane	*M. galloprovincialis*	Reduction of embryogenesis success	Beiras and Bellas (2008)
Endosulfan	*C. sapidus*	Inhibited hatching in higher tested concentrations	Lee and Oshima (1998)
	Crassostrea gigas	Increased abnormal D-larvae and the level of DNA strand breaks	Wessel et al. (2007)
	P. pugio	Increased hatching time	Wirth et al. (2001)
Synthetic pyrethroids			
Fenvalerate, cypermethrin	*C. sapidus*	Inhibited hatching	Lee and Oshima (1998)
Bifenthrin	*P. pugio*	Larval lethality	Harper et al. (2008)
Azoles			
Imazalil, triadimefon	*P. mammillata*	Decreased rate of fertilization, alterations of the anterior structures, incorrectly differentiated papillary nerves and nervous system	Pennati et al. (2006)
Phenylpyrazoles			
Fipronil	*P. pugio*	High larvae lethality	Key et al. (2007)
Triazines			
Atrazine	*P. pugio*	Non-toxic to larvae at tested concentrations	Key et al. (2007)
	Mercenaria mercenaria	Increased dry mass, higher condition index, dose-dependent mortality, higher shell major axis length	Lawton et al. (2006)
	Marisa cornuarietis	Delay in hatching	Sawasdee and Köhler (2009)
Benzoylphenylureas			
Diflubenzuron	*C. sapidus*	Inhibited hatching	Lee and Oshima (1998)
Terpenoids			
Methoprene	*C. sapidus*	Inhibited hatching in higher tested concentrations	Lee and Oshima (1998),
	P. pugio	No embryonic mortality at tested concentrations	Wirth et al. (2001)
Nitroguanidine nicotinoids			
Imidacloprid	*P. pugio*	Larvae lethality	Key et al. (2007)
	M. cornuarietis	Decreased heart rate	Sawasdee and Köhler (2009)

5 Role of Oxygen and Antioxidant Defenses in Embryogenesis

Despite intensive research, the mechanisms involved in teratogenesis are still not sufficiently understood, but it is known that they differ among various compounds. The following mechanisms by which xenobiotics may induce developmental effects, however, are known and include alterations of DNA (i.e., mutations, chromosomal aberrations, or nucleic acid metabolism effects), inhibition of specific enzymes, membrane alterations, modulation of cellular energy supplies, as well as disruption of retinoic acid signaling or oxidative stress (Beckman and Brent 1984; Wells et al. 2005). Most often, a complex set of factors and/or the accumulation of errors produces morphological malformations or embryo lethality (Meteyer 2000).

Oxygen plays a key role in metabolism and is critical to the early developmental stages of organisms. Several oxygen derivatives, known as reactive oxygen species (ROS), are known to have signaling functions and may affect several physiological and pathological processes in an organism (Covarrubias et al. 2008; de Lamirande and Gagnon 1995). At the level of embryogenesis, sensitive regulation of ROS has been linked to control of oocyte cleavage (Allen and Balin 1989), as well as oocyte maturation, ovarian steroidogenesis, ovulation, implantation, and formation of blastocysts (Guerin et al. 2001).

However, ROS levels must be continuously controlled to prevent them from becoming highly toxic to biological macromolecules (e.g., proteins, DNA, and membrane lipids) (Agarwal et al. 2003). The teratogenic potential of xenobiotics thus depends on embryoprotective pathways and on detoxification and macromolecule repair (Wells et al. 2005). General antioxidant defenses were recently shown to play an important role in protecting both early aquatic larval stages (Maria et al. 2009; Tilton et al. 2008) and later developmental phases, as well as the metamorphosis process (Dandapat et al. 2003).

To protect against ROS, cells contain both non-enzymatic antioxidant molecules (the ubiquitous thiol-containing tripeptide glutathione, vitamin E, and metallothioneins) (Tilton et al. 2008; Wiegand et al. 2001) and enzymes that can detoxify ROS. These enzymes include the following: superoxide dismutase (SOD; EC 1.15.1.1) that exists in mitochondria and cytosol, catalase (CAT; EC 1.11.1.6) that catalyzes removal of hydrogen peroxide in peroxisomes, and glutathione peroxidase (GPx, EC 1.11.1.9) present in the nucleus, mitochondria, and cytosol (Wang et al. 2002). Other important enzymes that are known to protect the embryo against reactive molecules are glutathione-*S*-transferase (GST; EC 2.5.1.18) (Anguiano et al. 2001; Peña-Llopis et al. 2003) and glucose-6-phosphate dehydrogenase (G6PD; EC 1.1.1.49) (Wells et al. 1997).

Antioxidants play an important role in protecting early larval stages against the effects of ambient oxygen levels in water (Arun and Subramanian 1998; Dandapat et al. 2003). Dandapat et al. (2003) showed that glutathione content increased during the metamorphic progression of the giant prawn larvae *Macrobrachium rosenbergii*. A similar increase was also observed in developing grass shrimp, *Palaemonetes pugio* (Winston et al. 2004), as well as in embryos of a toad, *Bufo arenarum* (Anguiano et al. 2001). In the trout, *Salmo iridaeus*, high CAT activities were observed during early development (in contrast to relatively lower levels of GPx), thus documenting the prime role of CAT over GPx in the removal of toxic hydrogen peroxide (Aceto et al. 1994). A gradual increase in CAT and GPx activities was observed during the progressive growth of the marine fish *Dentex dentex* from egg to larva (Mourente et al. 1999). On the other hand, in the same study, Mourente et al. (1999) showed that the titers of two other detoxification enzymes (GST and SOD) reached their highest levels in eggs, compared to later developmental stages. A gradual increase of antioxidant enzyme activities during embryogenesis, followed by a sudden rise in freshly hatched larvae, was also observed for the prawn *Macrobrachium malcolmsonii* (Arun and Subramanian 1998).

As is apparent, the results of available studies confirm that temporal changes of antioxidant agents are carefully regulated during the early development of aquatic organisms. Hence, such antioxidants play an important role. Notwithstanding, more research is needed to fully elucidate the physiological roles of ROS and antioxidant enzymes in maintaining homeostasis during early development.

6 Oxidative Stress in Embryotoxicity and Teratogenicity

While the previous section briefly described some physiological functions of ROS and the associated antioxidant defenses, the following paragraphs focus on the embryotoxicity that results from oxidative stress. Most research that has been conducted on this topic has included studies with model compounds such as hydrogen peroxide and has employed laboratory rodents or human embryos. In these studies, pro-oxidants induced severe oxidative stress damage to oocytes, mitochondrial alterations, ATP depletion, DNA damage and lipid peroxidation, apoptosis, or delays in whole embryo development (Aitken and Krausz 2001; Duru et al. 2000; Nasr-Esfahani et al. 1990; Ozolins and Hales 1999). The importance of oxidative stress in causing embryotoxicity or teratogenicity was also indirectly confirmed in mammalian and human studies, in which external additions of antioxidants prevented damage to embryos (Feugang et al. 2004; Fraga et al. 1991).

Similar to what occurs in mammals, studies with model pro-oxidants have also demonstrated detrimental effects in fish embryos and larvae (Westernhagen von 1988; Dietrich et al. 2005; Regoli et al. 2005), as well as in the larvae of the giant prawn *M. rosenbergii* (Dandapat et al. 2003). Moreover, the addition of antioxidants protected fish embryonal development against the effects of oxidative stress (Ciereszko et al. 1999; Toyokuni and Sagripanti 1992; Tilton et al. 2008).

7 Pesticides and Oxidative Damage During Early Development in Aquatic Organisms

Although pesticides may disrupt reproduction and development in many aquatic organisms (see Tables 1, 2, and 3), our search of the literature disclosed only a few studies that experimentally documented the role of oxidative stress in pesticide-induced teratogenicity (see Table 4).

Table 4 Teratogenic effects of some pesticides that are linked to effects on detoxification, antioxidative parameters, oxidative stress, or modulation of antioxidative processes in the early life stages of various fish species, amphibians, and invertebrates

Pesticide	Organism	Biochemical effect	Developmental effect	References
Fish				
Atrazine	*D. rerio*	Breakdown of the GST isoenzymes, altered microsomal and soluble GST activity, abnormal development, atrazine–GSH conjugate formation	Delay in embryonic development, unfinished epiboly or edema, decrease of heart rate and dysfunction of circulatory system, reduced development, reduced genesis of eyes, somites, otolithes, and melanophores	Wiegand et al. (2000, 2001)
Aldicarb, aldicarb sulfoxide	*D.rerio*	Inhibited carboxylesterase heart rate affection (embryo)		Küster and Altenburger (2007)
Paraquat	*O. mykiss*	Elevated G6PD and GR activity (juvenile)		Ĺkerman et al. (2003)
Azinphos methyl	*O. mykiss*	Reduced GSH level, CAT, and carboxylesterase activity (juvenile)		Ferrari et al. (2007)
Carbaryl	*O. mykiss*	Inhibited carboxylesterase and GSH level, altered CAT, induced GST activity and CYP1A level (juvenile)		Ferrari et al. (2007)
Dichlorvos	*S. aurata*	Increased LP level, decreased RNA/DNA ratio (juveniles)		Varó et al. (2007)
Endosulfan	*O. niloticus*	ROS production, lipid peroxidation (juveniles)		Tellez-Bañuelos et al. (2009)
Hexachlorobenzene	*C. carpio*	Altered GSH content and SOD and GPx, GR, GSSG activity, ROS generation, lipid peroxidation (juveniles)		Song et al. (2006)

Table 4 (continued)

Pesticide	Organism	Biochemical effect	Developmental effect	References
Amphibia				
Carbaryl	*B. arenarum*	Altered GSH content, SOD, GST, and GR activity, increased CAT and GPX activity	Progressive dropsy, body bending, and paralysis	Ferrari et al. (2009)
Azinphos methyl	*B. arenarum*	Decreased GSH level, altered GR, GPX, GST, and CAT, decreased SOD activity	Gill atrophy, notochord curvature, folded tail fin, generalized delay in the development, hyperactivity	Ferrari et al. (2009)
Parathion	*B. arenarum*	Alteration of GST activity	Decreased rate of gastrulas, curvature of the antero-posterior axis, tail folding, circle-swimming movement, frequent dropsy, and edema	Anguiano et al. (2001)
Malathion	*B. arenarum*	Reduced GSH content in both embryos and larvae, increased GST activity	Decreased rate of gastrulas, curvature of the antero-posterior axis, tail folding, circle-swimming movement, frequent dropsy, and edema	Anguiano et al. (2001)
	R. boylii	Decreased GR and CAT activities and the GSH pool (embryolarval)		Ferrari et al. (2008)
		Inhibited carboxylesterase activity, induced mixed function oxidase (larvae)		Venturino et al. (2001a, b)
		Depleted acid-soluble thiols (larvae) Larval mortality		Sparling and Fellers (2007)
Lindane	*B. arenarum*	Decreased GSH in embryo, increased GST activity	Decreased rate of gastrulas, irregular blastomeres, axis curvature, tail folding, edema, organ displacement, hemorrhage, hyperactivity, profuse scaling, dropsy, organ displacement, and bent tail	Anguiano et al. (2001)

Table 4 (continued)

Pesticide	Organism	Biochemical effect	Developmental effect	References
Dieldrin	*B. arenarum*	Increased GST activity	Exogastrulation, hyperactivity, hemorrhagia	Anguiano et al. (2001)
Acetochlor	*Bufo raddei*	Enhanced LP level and DNA single-strand break in liver (juvenile)		Liu et al. (2006)
Invertebrates				
Malathion	*C. gigas*	Increased CAT activity (larvae)		Damiens et al. (2004)
Heptachlor	*Homarus americanus*	Elevated CYP45 and HSP70 levels	Larvae mortality, delays in ecdysis	Snyder and Mulder (2001)
Carbofuran	*C. gigas*	Increased LP level, modulation of CAT activity (larvae)		Damiens et al. (2004)
Permethrin	*P. pugio*	Increased time to hatch, lethargy of larvae, altered GSH and LP levels (larvae)		DeLorenzo et al. (2006)

CAT, catalase; CoA, coenzyme A; CYP1A, cytochrome P 450 1A isozyme; EROD, ethoxyresorufin-*O*-deethylase; G6PD, glucose-6-phosphate dehydrogenase; GSH, glutathione; GSSG, oxidized glutathione; GR, glutathione reductase; GST, glutathione-*S*-transferase; GPx, glutathione peroxidase; HSP, heat-shock protein; LP, lipid peroxidation; ROS, reactive oxygen species; SOD, superoxide dismutase

Among the few pesticides on which oxidative stress effects were studied were the following: the triazine herbicide atrazine, the organophosphate insecticides parathion and azinphos methyl, and the organochlorine insecticides dieldrin and lindane.

Exposures of *D. rerio* embryos to atrazine lead to retardation of organogenesis (especially eyes, somites, otolithes, and melanophores), dysfunctions of the circulatory system, edemas, and a delay in embryonic development; in addition, these effects occurred in parallel with alterations of GST activities (Wiegand et al. 2000, 2001).

Mortality in embryos and developmental abnormalities, along with oxidative stress markers, were also observed in two studies with embryos of the toad *B. arenarum*. Anguiano et al. (2001) discovered that the organochlorine insecticide lindane caused abnormal segmentation of furrows, along with irregular blastomeres, profuse scaling, dropsy, organ displacements, and bent tail. Interestingly, only moderate alterations of embryonic morphology and hemorrhagia were observed after exposure to another organochlorine insecticide – dieldrin (Anguiano et al. 2001). In the same study, Anguiano et al. (2001) also showed that the organophosphate insecticides malathion and parathion were highly embryotoxic and caused a pathological curvature of the antero-posterior axis, tail folding edema, frequent dropsy, and also induced circle-swimming movements. Ferrari et al. (2009) studied the effects of carbaryl and azinphos methyl on the embryos of *B. arenarum* and demonstrated progressive dropsy, notochord malformations, gill atrophy, paralysis, and delayed development. The above-described effects were also correlated with modulations of glutathione levels and elevated activities of GST, SOD, CAT, and glutathione reductase (GR; EC 1.8.1.7) (Anguiano et al. 2001; Ferrari et al. 2009).

In studies with invertebrates, Snyder and Mulder (2001) demonstrated oxidative stress and pesticide toxicity after exposure to heptachlor or disruption of grass shrimp development by permethrin (DeLorenzo et al. 2006). Damiens et al. (2004) also showed larval toxicity and modulation of antioxidant and detoxification parameters after exposures to complex media contaminated with pesticides.

Direct toxic effects of pesticides on developing fish embryos were not found in other studies, but signs of oxidative stress and variable modulation of the antioxidative system were observed (Ĺkerman et al. 2003; Song et al. 2006; Ferrari et al. 2007; Varó et al. 2007; Küster and Altenburger 2007; Tellez-Bańuelos et al. 2009).

The bipyridyl herbicides paraquat and diquat are of special interest. The major mechanism by which they produce their toxic action in target organisms, whether animals or plants, is through lipid peroxidation. Disturbances of normal early developmental processes, after exposure to paraquat, were clearly documented to have occurred in *X. laevis* embryos (Vismara et al. 2000, 2001a; see Table 2). These toxic effects were prevented after the addition of the water-soluble antioxidant ascorbic acid to the test medium (Vismara et al. 2001b, 2006). Antioxidant protection by ascorbic acid was also confirmed in our studies (unpublished results), in which we compared the embryotoxicity of diquat and paraquat to *X. laevis*.

Few studies exist in which the oxidative stress damage caused by pesticides (herbicides atrazine, paraquat, and diquat; the organophosphate insecticides parathion and azinphos methyl; organochlorines dieldrin and lindane) to developing aquatic organisms has been described. Therefore, further research is needed in this area to better understand the levels of embryotoxicity that may result from other, less-explored pesticides.

8 Further Evidence – Pesticides and Antioxidative Defense in Adult Aquatic Biota

Although there are only a limited number of studies that link oxidative stress caused by pesticides with embryotoxicity, other evidence with adult aquatic organisms exists that supports the importance of this mechanism. Any comprehensive treatment of the topic of pesticide-induced oxidative stress in adults is beyond the scope of this chapter, and there are several credible recent reviews that address this topic (Valavanidis et al. 2006; Monserrat et al. 2006; Slaninova et al. 2009; Debenest et al. 2010). Nevertheless, to provide supporting data, representative studies that address selected pesticides (e.g., organophosphates, organochlorines, and bipyridyl herbicides) are presented in Tables 5, 6, and 7 (these tables address studies with fish, amphibians, and invertebrates, respectively).

In general, exposures of adult specimens to different pesticides induced antioxidative defenses, such as increases in titers of ethoxyresorufin *O*-deethylase (EROD), SOD, GST, and GR or G6PD, along with declines in glutathione concentrations and oxidative damage to lipids, DNA, proteins, and tissues (hepatic alterations, necrosis, etc.). For example, diazinon and glyphosate induced tissue-specific alterations of CAT and GPx activities, together with enhanced SOD activity and lipid peroxidation, in fish species (*Oreochromis niloticus* and *Prochilodus lineatus*; Durmaz et al. 2006; Langiano et al. 2008). Similarly, lipid peroxidation and detoxification responses were induced in methyl parathion-exposed *Brycon cephalus* (Monteiro et al. 2006), as well as in the mosquito fish *Gambusia affinis* that was exposed to the organophosphorus insecticides monocrotophos and chlorpyrifos (Kavitha and Rao 2008; Kavitha and Rao 2007, 2008). Glutathione redox cycle and CAT were shown to protect against endosulfan-induced toxicity in trout *Oncorhynchus mykiss* cells (Dorval et al. 2003; Dorval and Hontela 2003). Endosulfan also induced lipid peroxidation and altered various enzymatic activities in *Jenynsia multidentata* (Ballesteros et al. 2009). Paraquat, a herbicide that acts via ROS production, induced lipid peroxides and modulated SOD, GR, and GST in various fish, such as *Sparus aurata* (Pedrajas et al. 1995; Rodríguez-Ariza et al. 1999) or Nile tilapia *O. niloticus* (Figueiredo-Fernandes et al. 2006a, b). Paraquat also induced protein carbonylation in liver, kidney, and gills and modulated glutathione and ascorbic acid levels in *Channa punctata* (Parvez and Raisuddin 2005, 2006).

Comparable results were obtained in experiments with the amphibian (frog) *Rana ridibunda* (Table 6). Herein, mixtures of propamocarb and mancozeb caused elevated lipid and protein peroxidation and suppressed SOD activity (Falfushinska et al. 2008). Oxidative stress caused by pesticides, pyrethroid insecticides for example, was also observed in several experiments performed with invertebrates

Table 5 The effects of selected pesticides on detoxification, antioxidative, and other important biochemical parameters in fish

Pesticide	Organism	Biochemical effect	References
Carbamates			
Molinate	*Anguilla anguilla*	Increased hepatic GSH, GR and GSH:GSSG ratio, decreased muscular GSH	Peńa-Llopis et al. (2001)
Carbaryl	*O. niloticus*	Decreased SOD, GR, GST, and CAT activity, hepatocellular basophilia, necrotic foci	Matos et al. (2007)
Organophosphates			
Monocrotophos	*G. affinis*	SOD, CAT, GR, and lipid peroxidation induction, recovery response of antioxidant enzymes	Kavitha and Rao (2007)
Chlorpyrifos	*G. affinis*	SOD, CAT, and GR inhibition, recovery response of antioxidant enzymes, lipid peroxidation	Kavitha and Rao (2008)
Azinphos methyl	*O. niloticus*	Increased activity of G6PD, GPx, and GR, SOD decrease	Oruçand Üner (2000)
	C. carpio	Increase of SOD and GST activities, elevation in CAT and GPx activities in carp decrease of GPx in tilapia	Oruç et al. (2004)
Glyphosate	*P. lineatus*	Plasma glucose and CAT activity increase, histological alterations impairing normal organ functions	Langiano and Martinez (2008), Modesto and Martinez (2010)
		Reduced SOD and GPx activities, increased GST activity and GSH level, lipid peroxidation	
	Carassius auratus	Decreased GSH content and SOD, GR, G6PHD activity and hepatic GST, increased CAT activity	Lushchak et al. (2009)
Diazinon	*O. niloticus*	SOD increase, CAT and GPx alteration, lipid peroxidation	Durmaz et al. (2006)
	O. mykiss	Increased LP level, GSH depletion, modulation of SOD, GR, GST, GPX activities	Isik and Celik (2008)
	C. carpio	Increased SOD, decreased CAT, and altered GPx activity and protein carbonyl level, lipid peroxidation	Oruçand Usta (2007)

Table 5 (continued)

Pesticide	Organism	Biochemical effect	References
Methyl parathion	*Brycon cephalus*	SOD, CAT, GST induction, GPx alteration, lipid peroxidation	Monteiro et al. (2006)
	O. mykiss	Increased LP level, GSH depletion, modulation of SOD, GR, GST, GPx activities	Isik and Celik (2008)
Malathion	*S. aurata*	Increased LP and GST	Pedrajas et al. (1995)
Fenthion	*O. niloticus*	Increased GSH and GSSG content and GPx activity	Piner et al. (2007)
Organochlorines			
Endosulfan	*O. mykiss*	CAT, GPx, GSH alterations, lipid peroxidation	Dorval and Hontela (2003)
	J. multidentata	Reduced GSH and GPx, induced GST and CAT, lipid peroxidation	Dorval et al. (2003)
	C. punctata	GST, GR, GPx, and CAT alterations, lipid peroxidation	Ballesteros et al. (2009)
		Increased protein carbonyls in liver, kidney, and gills	Parvez and Raisuddin (2005)
Dieldrin	*S. aurata*	CAT, SOD, and palmitoyl-CoA-oxidase induction, increased protein concentration in peroxisomal fraction; increased LP and GST;increased 8-oxoG marker	Pedrajas et al. (1995, 1996), Rodríguez-Ariza et al. (1999)
Chlorothalonil	*Morone saxatilis*	ROS production, altered GSH level	Baier-Anderson and Anderson (2000)
DDT	*Hoplias malabaricus*	Increased intracellular ROS, increased CAT and G6PDH activities, GSH content, lipid peroxidation and protein carbonyl level, decreased SOD, GST, and GR activities; decreased cell viability	Filipak Neto et al. (2008)
Triazines			
Atrazine	*D. rerio*	Induced cytochrome P450 content, increased NADPH-P450 reductase, erythromycin *N*-demethylase, and aminopyrine *N*-demethylase	Dong et al. (2009)
	Lepomis macrochirus	Increased hepatic GSH and GSSG levels and GST, SOD activities, lipid peroxidation, altered GPx and GR activities	Elia et al. (2002)

Table 5 (continued)

Pesticide	Organism	Biochemical effect	References
Mixture of atrazine, simazine, diuron, isoproturon	*C. auratus*	Enhanced production of superoxide, induced SOD activity in liver, reduced CAT activity	Fatima et al. (2007)
Simazine	*C. carpio*	Lipid peroxidation, increased GSH level	Oropesa et al. (2009)
Bipyridyls			
Paraquat	*O. niloticus*	High hepato-somatic and gonado-somatic index, increased EROD activity, hepatic alterations of parenchyma, like vacuolization, necrosis, and an increase of macrophage aggregates and eosinophilic granular cells; higher SOD, GST activity, gender-dependent increase of GR	Figueiredo-Fernandes et al. (2006a, b)
	C. punctata	Increased protein carbonyls in liver, kidney, and gills, reduced GSH in liver and gills, increased ascorbic acid level	Parvez and Raisuddin (2005, 2006)
	S. aurata	Increased LP, GST, and SOD Decreased GPX, higher G-6PDH activity, increased GR	Pedrajas et al. (1995), Rodríguez-Ariza et al. (1999)
2,4-D-Phenoxy carboxylic acid			
2,4-Dichlorophenoxy acetic acid dimethyl amine	*O. niloticus*	Increased activity of G6PD, GPx, and GR	Oruç and Üner (2000)
	C. carpio	Increase of SOD and GST activity, elevation in CAT and GPx activities in carp decrease of GPx in tilapia	Oruç et al. (2004)
Synthetic pyrethroids			
Deltamethrin	*C. punctata*	Lipid peroxidation and GSH level increase, alterations in CAT, SOD, and GST activity and ascorbic acid level	Sayeed et al. (2003), Parvez and Raisuddin (2005)
		Increased protein carbonyls in liver, kidney, and gills	
Cypermethrin	*C. carpio*	Increased hepatic SOD and CAT activities, decreased GPx, lipid peroxidation	Uner et al. (2001)
	O. niloticus	Increased hepatic SOD, GPx, and CAT activities, lipid peroxidation	

Table 5 (continued)

Pesticide	Organism	Biochemical effect	References
Bifenthrin	*Cyprinodon variegatus*	Increasing trend in GSH level and CAT activity with toxicant dose	Harper et al. (2008)
Chloroacetanilides			
Butachlor	*C. gariepinus*	Lipid peroxidation, altered SOD, GST, and CAT activities and GSH level	Farombi et al. (2008)
Organofluorine			
Etoxazole	*O. niloticus*	Lipid peroxidation	Sevgiler et al. (2004)
Pyrazoles			
Fenpyroximate	*Paralichthys olivaceus*	Altered SOD, CAT, GST, GPx, and EROD activities	Na et al. (2009)
Dichlorobenzenes			
3,4-Dichloroaniline	*C. auratus*	Enhanced SOD activity and LP, decreased NO synthase and GSH level in liver	Li et al. (2003)

CAT, catalase; EROD, ethoxyresorufin-*O*-deethylase; G6PD, glucose-6-phosphate dehydrogenase; GSH, glutathione; GSSG, oxidized glutathione; GR, glutathione reductase; GST, glutathione-*S*-transferase; GPx, glutathione peroxidase; NADPH P450, NADPH–cytochrome P450 reductase; NO, nitric oxide; LP, lipid peroxidation; SOD, superoxide

Table 6 Effects of carbamates on detoxification, antioxidative, and other important biochemical parameters in amphibians

Pesticide	Organism	Biochemical effect	Reference
Amphibians			
Carbamate			
Mixture of propamocarb and mancozeb	*R. ridibunda*	Decreased SOD; lipid and protein peroxidation, neurotoxicity and endocrine disruption	Falfushinska et al. (2008)

SOD, superoxide dismutase

(Table 7). Several studies were conducted with mollusks on the pyrethroid insecticides, for example, cypermethrin and alphamethrin; these insecticides inhibited reproduction and induced oxidative stress in the freshwater snail *Lymnaea acuminata* (Tripathi and Singh 2004). In addition, lindane, an organochlorine insecticide, induced oxidative damage and stress responses in the mussel *Mytilus galloprovincialis* (Khessiba et al. 2005).

Table 7 The effects of selected pesticides on detoxification, antioxidative, and other important biochemical parameters in aquatic invertebrates

Pesticide	Organism	Biochemical effect	References
Organophosphates			
Fenitrothion	*M. galloprovincialis* *Flexopecten flexuosus*	GSH and GSSG depletion, reduction of GSH/GSSG ratio, decreased survival	Peña-Llopis et al. (2002)
Organochlorines			
Lindane	*M. galloprovincialis*	CAT activity induction	Khessiba et al. (2005)
Endosulfan	*Penaeus monodon*	Increased HSP level in muscles	Dorts et al. (2009)
Tetradifon	*D. magna*	Decreased protein, lipid, glycogen, and caloric content, decreased mean body dry weight	Villarroel et al. (2009)
Synthetic pyrethroids			
Cypermethrin alphamethrin	*L. acuminata*	Altered oxidative metabolism in hepatopancreas and ovotestis tissues, reduced survival	Tripathi and Singh (2004)
Deltamethrin	*P. monodon*	Increased LP level and protein carbonyls in gills	Dorts et al. (2009)
Bipyridyls			
Paraquat	*Biomphalaria glabrata*	Increased LP, decreased SOD	Cochón et al. (2007)

CAT, catalase; GSH, glutathione; GSSG, oxidized glutathione; HSP, heat-shock protein; LP, lipid peroxidation; SOD, superoxide dismutase

9 Summary

Many pesticides have been documented to induce embryotoxicity and teratogenicity in non-target aquatic biota such as fish, amphibians, and invertebrates. Our review of the existing literature shows that a broad range of pesticides, representing several different chemical classes, induce variable toxic effects in aquatic species. The effects observed include diverse morphological malformations as well as physiological and behavioral effects. When developmental malformations occur, the myoskeletal system is among the most highly sensitive of targets. Myoskeletal effects that have been documented to result from pesticide exposures include common notochord and vertebrate column degeneration and related abnormalities. Pesticides were also shown to interfere with the development of organ systems including the eyes or the heart and are also known to often cause lethal or sublethal edema in exposed organisms. The physiological, behavioral, and population endpoints affected by pesticides include low or delayed hatching, growth suppression, as well as embryonal or larval mortality. The risks associated with pesticide exposure increase particularly during the spring. This is the period of time in which major pesticide applications take place, and this period unfortunately also coincides with many sensitive reproductive events such as spawning, egg laying, and early development of many aquatic organisms.

Only few experimental studies with pesticides have directly linked developmental toxicity with key oxidative stress endpoints, such as lipid peroxidation, oxidative DNA damage, or modulation of antioxidant mechanisms. On the other hand, it has been documented in many reports that pesticide-related oxidative damage occurs in exposed adult fish, amphibians, and invertebrates. Moreover, the contribution of oxidative stress to the toxicity of pesticides has been emphasized in several recent review papers that have treated this topic.

In conclusion, the available experimental data, augmented by several indirect lines of evidence, provide support to the concept that oxidative stress is a highly important mechanism in pesticide-induced reproductive or developmental toxicity. Other stressors may also act by oxidative mechanisms. This notwithstanding, there is much yet to learn about the details of this phenomenon and further research is needed to more fully elucidate the effects that pesticides have and the environmental risks they pose in the early development of aquatic organisms.

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Mixtures of Environmental Pollutants: Effects on Microorganisms and Their Activities in Soils

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1 Introduction

The presence of unwanted substances in the environment becomes “pollution” when damage or deleterious effects caused by those substances are detected. Pollution is essentially a process by which a natural or a man-made resource becomes unfit for beneficial use (Enger and Smith 2006). Many man-made substances, in addition to certain naturally occurring organic and inorganic compounds, different forms of energy (e.g., heat, light and noise), and other things, add to the diversity of pollutants. Pollutants, which can occur at the local or the global level, render varying influences on soil and water that depend on the nature and spreading rate of the pollutants themselves, and produce short- or long-term effects on elements of an ecosystem. Undesirable changes in air, water, and soil may induce diverse responses from living organisms that are similar to those induced by the presence of pollutants. The effects on organisms caused by pollutants may range from innocuous to toxic. Toxicity, which has been defined as an inherent property of a substance to cause an adverse biological effect, is the result of a chemical disturbance that affects complex and interrelated systems involving cells, tissues, organs, or metabolic processes (ECETOC 1985).

An important driver in the process of pollution is the exposure to and response of the individual organism to a pollutant or a pollutant mixture. In soil environments, pollution results from either intentional (waste disposal) or unintentional application of either a single chemical or complex mixtures of chemicals such as industrial effluents, municipal waste, sewage sludge, or others (Table 1). The presence of a

Table 1 Co-contaminated sites that have probable inorganic and organic pollutants present in man-made ecosystems

Contaminated site	Nature of pollutant mixtures
Pest management	
Agricultural soils	Cadmium (Cd), insecticides, herbicides, fungicides
Vineyard soils	Copper (Cu), pesticides
Gas work-site soils	
Defense sites	Chlorinated solvents [trichloroethene (TCE), perchloroethylene (PCE), chloroform, etc.] and metals (Cu, Cd, etc.)
Cattle/sheep dip sites	Arsenic, dichlorodiphenyltrichloroethane (DDT) and other insecticides
Pesticide manufacturing and storage facilities	Pesticides and other degradation products (for example, DDT and its metabolites; hexachlorocyclohexane (HCH) and its carriers)
Petroleum storage sites	Petroleum hydrocarbons and lead (Pb)
Landfills	Cocktail of various pollutants
Mine site soils	Metal mixtures
Biosolid applied soils	Organics and metals
Sewage sludge	Mixture of organic and inorganic chemicals

single pollutant is often characterized by reliable analytical techniques, whereas pollutant mixtures that have variable composition are rarely adequately chemically characterized because of their complexity and the deficiencies that exist in analytical techniques. Mixtures of chemical substances are generally defined as being either (a) simple mixtures containing "two or more identifiable components but few enough that the mixture toxicity can be adequately characterized by a combination of the component toxicities and the component interactions" or (b) complex mixtures containing "so many components that any estimation of toxicity based on components' toxicities contains too much uncertainty and error to be useful" (US EPA 2000). Most scientific studies have so far been focused on the effects of exposures of individual chemical substances to a single or multiple organisms (Yang 1994; Monosson 2005).

The biological response to pollutants is varied, because living organisms differ in their sensitivities to the same substance, and relative sensitivity depends on the duration and level of exposure to the pollutant. In an ecosystem, the adverse effects of pollutants can be apportioned among a myriad of organisms ranging from microorganisms to humans. Although microorganisms are of minuscule size, their numbers and metabolic versatility allow them to play a very significant role, especially in the soil and aquatic environments. As early as 1976, Atlas et al. reported that incubation of water from coastal ponds along Prudhoe Bay that had been contaminated with Prudhoe crude oil led to several changes that included the following: an increase in bacterial populations by several orders of magnitude, replacement of amoeboid protozoans by flagellated protozoans, disappearance of coccoid green algae, and an increase in diatoms. Although microorganisms are affected by pollutants, the microorganisms themselves can accelerate the rates of degradation of many environmental pollutants. The scientific knowledge gained on how microorganisms interact with individual chemical pollutants over the past decades has helped to abate environmental pollution. Nevertheless, the ever-increasing production and accumulation of pollutants and their wastes, as a consequence of the human population explosion, has accelerated efforts to improve the quality and quantity of life in terms of food and energy production (Jouany 1998). The creation, introduction, and management of pollutants in the environment will continue to remain a contentious global issue. With the dwindling resource base, concerted research efforts to reduce the adverse effects that result from pollution are more necessary now than ever before.

Traditionally, chemical pollution remains localized or is diffused from a point source. Around the source, a well-defined chemical substance or cocktail of chemical substances normally become distributed to form a gradient. Such diffuse pollution is often characterized as coming from many sources and having erratic distribution, which can lead to a complex mixture of discontinuous low-to-moderate concentrations over a large area. Whether localized or diffuse, mixtures of chemical substances show spatial heterogeneity and "hot spots" of individual chemical pollutants may exist at levels that are highly toxic. Although there are many naturally occurring toxic chemical substances, the production of chemical substances of anthropogenic origin for sale [e.g., cadmium as a pollutant in the European Community (van der Voet et al. 1994)] and intentional application or use of them pose serious pollution threats. Another example of man-made pollution is soil contamination resulting from disposal of wood preservative wastes, coal tar, or airborne combustion by-products, all of which may pose genotoxic hazards. Genotoxicity and carcinogenicity are often considered to be intrinsic properties of many chemical pollutants. Unfortunately, information on the carcinogenicity of most chemical pollutants is limited; the Carcinogenic Potency Database retains data from long-term rodent cancer tests for only about 1,547 chemicals (Gold 2010).

Having reliable safety and occurrence data on environmental pollutants is critical. Unfortunately, analyzing the biological effects from exposure to environmental pollutants in the laboratory has limitations. Synergistic or antagonistic interactions that may exist at polluted sites are difficult to reproduce in a laboratory. Therefore, biological effects that are measured at or near a polluted site generally produce more appropriate and useful information on the effects caused by the pollution. Moreover, microorganisms that reside in these sites can serve as useful biomarkers of environmental pollution. A perusal of the literature on the nontarget effects of environmental pollutants indicates that the data available are predominantly concerned with individual chemical substances (Venkateswarlu 1993; Ramakrishnan et al. 2010), despite the common and widespread occurrence of environmental pollutants as mixtures (organic–organic, organic–inorganic, inorganic–inorganic, and parent compounds and their metabolites). The primary purpose of this review is to evaluate the effects of chemical pollutants, particularly pollutant mixtures, on microorganisms and microbial processes.

2 Occurrence of Pollutants

Pollution, the term derived from “pollute” which means “to foul,” occurs in many ways. Both natural and anthropogenic activities generate various polluting substances that may affect air, soil, and/or water. Pollution may emanate from various sources, such as industrial, agricultural, community activities and/or effects of human residential density. When pollutants enter the environment, potentially disastrous effects can result. Below, we address the nature of both intentional and accidental pollutants.

2.1 Intentional Pollution

Intensive anthropogenic activities and utilization of many natural resources may produce continuous waste generation and eventual pollution of the environment. Traditionally, waste is disposed of by incineration or removal to landfills. Intentional disposal in landfills, lagoons, or surface impoundments is considered to be a cost-effective waste disposal strategy and is based on the principle that soils have the capacity to retain and degrade pollutants. But, disposal in landfills, or at similar sites, has the serious disadvantage of being a possible source of pollutant migration to groundwater and/or to the atmosphere. Widespread disposal of wastes, fossil fuel combustion, and solid waste incineration has contributed to regional soil contamination. In contrast, an alternative is to use municipal waste as organic fertilizing materials, which can modify and improve soil conditions by enhancing the levels of organic matter, total nitrogen, available phosphorus, exchangeable bases, CEC (cation exchange capacity), and some trace elements (Olowolafe 2008).

The impact of pollutants varies enormously. For example, eutrophication of water bodies resulting from nutrient run-off from agricultural fields and sewage can encourage profuse growth of undesirable algae with a potential adverse impact on the ecosystem. Since the biological oxygen demand (BOD) is much affected by eutrophication, it is, itself, considered to be pollution. When exposed to certain polluting elements or substances, some microorganisms accumulate them and thus produce high body loads relative to the external concentrations. Microorganisms are also considered to be unique agents because of their ability to degrade diverse classes of chemical substances (Alexander 1999). However, not all novel chemical substances synthesized by man are expediently used as substrates for growth by microorganisms. When discharged in unusually high amounts, these substances can persist in the environment. Moderately hydrophobic substances, such as polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethanes (DDTs), have octanol-water partition coefficients (K_{ow}) between 100 and 100,000, are persistent and bioaccumulative chemical substances, and are poorly metabolized. These substances magnify in aquatic food webs and exhibit a high degree of bioaccumulation in food webs containing air-breathing animals because of their high octanol–air partition coefficient (K_{OA}) and corresponding low rate of respiratory elimination to air (Kelly et al. 2007). Thus, the selective action of pollutants influences different species in different ways, to different extents, or at different concentrations, with resultant alterations in the productivity and recycling within the environment.

Many chemical substances present, even at low levels, can exhibit long-term persistence in the environment. When applied in excess, even macronutrients such as N and P, and organic pesticides have short-term detrimental effects on environmental water quality. Pesticides are intentionally applied and are used worldwide in agriculture to protect crops from pests and in public health to control diseases that are transmitted by vectors or intermediate hosts. Similarly, pharmacologic agents, intentional food additives, or processing agents are used extensively for the health and comfort of human and animals, and these may reach the environment. Walraven and Laane (2009) reviewed the riverine discharge of individual pharmaceuticals and discovered that they varied from less than the limit of detection (LOD) to 27 t year^{-1} along the Dutch Coastal Zone in 2002. The long-term and steady increase in the use of pharmaceuticals has led them to attain the status of environmental pollutants (Toft et al. 2004). Inputs of contaminating heavy metals and other trace elements to agricultural lands from application of soil amendments or persistent agrochemicals pose a long-term threat because of their indefinite residence time (Hesterberg 1998). The fate of many soil pollutants is governed by multiple factors: the type of the contaminant (e.g., heavy metal cations, oxyanions, and pesticides), their solubility and soil mobility, and the mechanisms by which they bind in soils. The binding rates in soil of such agents are affected by the relative matrix composition, mineralogy and organic matter content, soil heterogeneity, pH, redox potential, and variations in dissolved organic matter concentration.

2.2 Accidental Pollution

Accidental leaks/spills occurring during transport and storage of industrial materials or agricultural chemicals have polluted areas that were never intended as sites for waste disposal. Large-scale fires or spills at chemical warehouses and plastic storage facilities have resulted in pollution from agents such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs) and biphenyls (PCBs), and substituted benzenes, amongst others (Meharg 1994a, b). In the Arabian Gulf and along other shorelines, chronic or severe pollution by hydrocarbons proceeds mainly from natural oil seepage, accidental damage to pipelines, accidental spillage from tankers, or tanker deballasting (Massoud et al. 1996). Such accidental releases of petroleum products, which are complex chemical mixtures, have contaminated thousands of sites (Tonner-Navarro et al. 1998). The chemical properties of the agents involved determine the extent of effects that are produced from the accidental release of most toxic chemicals (Bubbico and Mazzarotta 2008).

There are also cases of unintentional, long-term application of pollutants to the environment. For instance, cadmium is a pollutant of agricultural soils and has a strong bioaccumulative tendency as a result of its chemical similarities to zinc. Cd reaches agricultural soils as an impurity in phosphate fertilizers (Goodroad and Caldwell 1979). Huang et al. (2005) reported that nearly all of the arsenic and lead that are added to the environment from the application of iron and zinc fertilizers remained in soils. The long-term use of copper-based chemical substances has resulted in increased soil copper concentrations of 29–131 mg kg^{-1} in India (Prasad et al. 1984) and 11–320 mg kg^{-1} in Australia (Wightwick et al. 2008). Similarly, the long-term use of fungicidal sprays (Merry et al. 1986) and soil application of sewage sludge (Keefer et al. 1986) have led to elevated soil levels of arsenic, lead, and other metals. Thus, the long-term use of many chemical substances, albeit at low concentrations, can result in pollution of great concern.

3 Mixtures of Pollutants

The term "chemical substance" means "any organic or inorganic substance of a particular molecular identity, including (i) any combination of such substances occurring in whole or in part as a result of a chemical reaction or occurring in nature and (ii) any element or uncombined radical" (US EPA 2010). Almost all substances are mixtures and only a few pure substances are found in nature, and that applies to pollutants as well. Mixtures contain more than one chemical substance (two or more elements or compounds that are in close contact) and are homogenous in that they have a uniform composition throughout their mass, or are heterogeneous, and lack a uniform composition throughout their mass. In soil environments, exposure to pollutants occurs from either a single chemical substance or complex mixtures such as industrial and agricultural chemicals, and wastes from industries and municipalities (Mumtaz 1995).

3.1 Organic Pollutant Mixtures

Several aliphatic, alicyclic, or aromatic compounds are products, intermediates, or by-products of the chemical and pharmaceutical industries and reach the soil or aquatic environment by manifold means and from manifold sources. Generally, organic pollutants are thermodynamically unstable under dynamic soil conditions and are ultimately converted to less toxic or harmless products by physico-chemical action (such as photodecomposition, partitioning, volatilization, and leaching) and biological action (such as microbial metabolism, plant uptake, and release) (Stone 1991; see Fig. 1). But the levels of many degradable organic chemical substances become elevated if they are applied repeatedly over short periods at high concentrations. There are also various recalcitrant organic compounds that are persistent in soil and water environments, and these may pose serious environmental hazards.

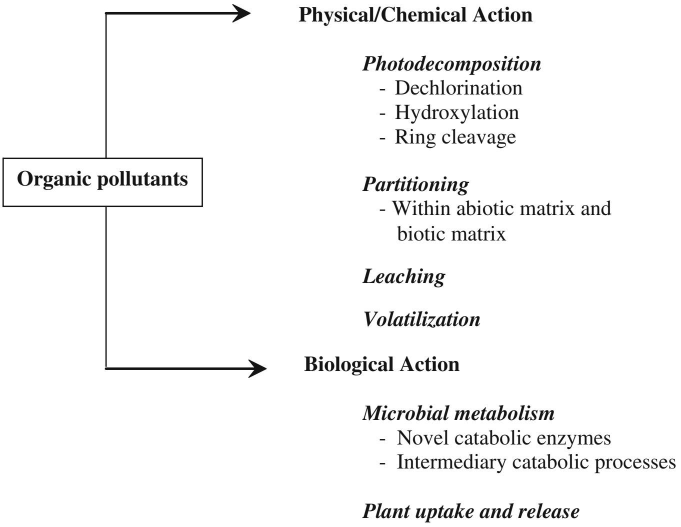

Fig. 1 Dissipation pathways for organic pollutants in soil and water environments

Because the concentrations of the persistent organic pollutants (POPs) commonly measured in soils are significantly correlated with those that occur in the atmosphere, Vogt et al. (1987) regards the POP concentrations in soils to be a good indicator of the proximity of pollution sources and levels of air pollution. Unfortunately, many organic chemical pollutants are neither monitored nor require any reduction prior to their disposal in soils (Harrison et al. 2006). The co-occurrence of certain organic chemical pollutants is very common at contaminated sites. For example, methyl *tert*-butyl ether (MBTE) commonly occurs with other organics such as BTEX (benzene, toluene, ethylbenzene, and xylene) at contaminated sites. MBTE, which is manufactured by chemically reacting methanol and isobutylene, can easily pollute both surface and groundwater if there are spillages. Because of its high water solubility, low adsorption to soil, and low biodegradation rate, MTBE is a common groundwater contaminant in the United States (US EPA 2006).

The POPs of global concern include PAHs, PCBs, and organochlorine pesticides (OCPs). PAHs are the by-products from incomplete combustion or pyrolysis of organic substances like coal, oil, petrol, and wood. PAHs originate from two main anthropogenic sources: fossil fuel combustion and release of petroleum products. Certain natural processes can also result in the presence of PAHs at the surface of agricultural soils (Blumer 1961). Long-term flooding, irrespective of soil type, can lead to an increase in the soil content of PAH (Oleszczuk et al. 2007). Nevertheless, anthropogenic PAHs predominate in agricultural and forest soils of temperate and tropical regions (Manz et al. 2001; Wenzel et al. 2002). Total PAH concentrations range from 1.56 to 90.44 μg g^{-1} in highly polluted areas (Shiaris and Jambard-Sweet 1986; Nishigima et al. 2001; Muniz et al. 2004). In several sites across the Seine basin of France, the concentrations and congener profiles for PAHs (Σ14 PAHs) and PCBs (Σ7 PCBs) range from 450 to 5,650 μg kg^{-1} and 0.09 to 150 μg kg^{-1}, respectively; these pollutants displayed spatial differences and gradients with a ratio of up to one order of magnitude for PAHs and two orders of magnitude for PCBs (Motelay-Massei et al. 2004). An example of a natural or an anthropogenic source of PAHs is creosote. Creosote is primarily used as a wood preservative. Creosote is obtained from beech trees and other woods after high temperature treatment, the Creosote Bush produces a resin containing creosote, and creosote is obtained from coal tar that is distilled from crude coke over tar. Coal tar–creosote is a mixture of thousands of chemical substances; 85% is comprised of 2–5-ring PAH compounds, along with certain phenols, and cresols and all of these can contaminate soil and groundwater (Conrad et al. 1999). The compounds with four or more aromatic rings are recalcitrant and can be mutagenic.

Often, components of the organic mixtures are not fully chemically characterized. The contamination of heavy metals and petroleum hydrocarbons in the sediments of Montevideo Harbor, Uruguay, comprised an unresolved complex mixture (UCM) of about 76% of a total aliphatic fraction; levels detected ranged from 21 to 120 μg g^{-1} (Muniz et al. 2004). At sites that have chronic degraded oil contamination, the UCM of branched and cyclic hydrocarbons is a common feature. In fact, the levels of UCM detected provide evidence for long-term petroleum contamination (Gough and Rowland 1990). Recently, Wang et al. (2008) also suggested that high mountains may serve as “cold traps” for POPs. The detection of DDT (1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane), HCHs (hexachlorocyclohexanes), and PAHs in ice core samples from East Rongbuk Glacier provided a long-term record of the atmospheric deposition of these pollutants. The extremely low solubility of OCPs fosters their escape to the solution phase, wherein they can adhere to lipophilic substances present or gradually volatilize into the atmosphere. The indiscriminate disposal of many OCPs and their associated wastes has led to large-scale occurrences of highly contaminated soils worldwide (Harner et al. 1999; Wang et al. 2009).

3.2 Inorganic Pollutant Mixtures

Inorganic pollutants are not usually biodegradable and can only be transformed from one chemical state to another. Inorganic salts are comprised of basic and acidic radicals and these can be present as mixtures. In soils, minerals and metals typically arise from the net effects of geological and soil-forming processes of elements (Kabata-Pendias and Adriano 1995). The individual concentrations of minerals and metals are governed by several factors: the nature of their parent materials, climate, topography, human activities, etc. A mineral is an element or a chemical compound that is normally crystalline and has been formed through geological processes; some minerals identical to natural ones are formed through anthropogenic activities, and these are referred to as synthetic equivalents. Heavy metals or metalloids are major inorganic pollutants and may be deposited in the atmosphere or soil due to industrial activities (e.g., smelting). Heavy metals or metalloids are major inorganic pollutants and may be deposited in the atmosphere, may leach to groundwater, and may or may not be degraded (Martley et al. 2004).

Inorganic pollutants may derive from natural and/or anthropogenic sources, and may be local or regional in scope. For example, mercury is naturally emitted, primarily from volcanoes, and is anthropogenically emitted from coal-fired power plants (Hylander and Goodsite 2006). All forms of mercury are toxic, and this element has no known biological benefits. Inorganic pollution can be serious, even when it occurs from a seemingly simple human activity. For example, using groundwater for drinking and agricultural purposes has led to widespread arsenic pollution of water in several countries, and in Bangladesh and India, in particular. Arsenic toxicosis is known to affect millions of individuals (Bhattacharya et al. 2007). The bioavailability of an element to living organisms depends on its chemical speciation (De Haan and Visser-Reyneveld 2004). Therefore, data on total concentrations of inorganic pollutants alone cannot provide precise information on the propensity to be bioavailable or toxic.

Most industrial wastes contain mixtures of metals and nonmetals at varying concentrations. Harris and Ragusa (2000) characterized the acid drainage from Nairne Pyrite Mine (AMD), South Australia, and discovered that it contained several inorganic chemical substances at concentrations exceeding the Australia and New Zealand Environment and Conservation Council (ANZECC) water quality guideline values. Many inorganic pollutants that occur in wastes that are applied to soil, even at very low concentrations, can accumulate in soils over the long term. Known examples at several worldwide sites include cadmium pollution of agricultural soils from long-term use of phosphate fertilizers (Goodroad and Caldwell 1979) and soil pollution from arsenic, lead, and other metals through application of sewage sludge, in which they exist as contaminants (Keefer et al. 1986).

3.3 Inorganic and Organic Pollutant Mixtures

The Registry of American Chemical Society's Chemical Abstracts Service (CAS) has indexed nearly 23 million organic and inorganic compounds (CAS 2008). Over seven million compounds are commercially available. But the "Priority Pollutant List" of the US Environmental Protection Agency (USEPA) contains a mere 126 compounds, and the "Priority List of Hazardous Substances" of the Agency for Toxic Substances and Disease Registry (ATSDR) has only 253 substances (ATSDR 2008). The sources of pollutants of inorganic and organic combinations can be natural and anthropogenic. For example, crustacean carapaces, mollusk shells, bone and teeth tissues in vertebrates are naturally occurring organic–inorganic composites. Many manufactured organometallic derivatives are marketed worldwide. Such derivatives include fungicides, miticides, molluscicides, nematocides, ovicides, rodent repellants, wood preservatives, and antifouling paints (Hoch 2001). Both the products and waste materials from the industries that produce such agents reach the soil environment. Paints are inorganic pigments suspended in organic mixtures and are one of the oldest and most widely used organic–inorganic industrial agents. As mentioned above, fungicides may be important organic–inorganic composites. One example, mefenoxam, an acylanilide (R-isomer of metalaxyl) fungicide used to control foliar or soil-borne fungal diseases caused by *Phytophthora,* is applied either alone or in combination with other fungicides. As an additional active ingredient, copper is often used in combination with mefenoxam to accentuate fungicidal action (Russell 1995). Wide use of such combinations may lead to the creation of many diffuse pollutants.

Soils that are contaminated with PAHs often contain high amounts of other pollutants such as heavy metals, which are often derived from the same contamination source (van de Leemkule et al. 1998; van Hesteren et al. 1998). Cattle dip sites in Australia are known to be contaminated with both arsenic and DDT residues (Edvantoro et al. 2003). More recently, many hybrid organic–inorganic nanocomposites have entered development for use in smart membranes, new catalysts, and sensors; other nanocomposites are finding use in intelligent therapeutic vectors that have controlled release properties. These hybrid materials will increasingly pose additional challenges to pollutant disposal and environmental degradation.

4 Analysis of Soil Pollutant Mixtures

Pollutant characterization involves first detecting the individual pollutants and then quantifying them by an appropriate technique, the nature of which will depend on the chemical form and pollutant levels involved. In recent years, comprehensive national and international standards for pollutant monitoring have been established.

Table 2 Chemical characteristics of polluted acid waste from Nairne Pyrite Mine Acid Mine Drainage, South Australia vs. some quality criteria for water for the Australia and New Zealand Environment and Conservation Council (ANZECC)

	Concentration (mg L^{-1})	
Component	Acid drainage	Guideline
Aluminum	46	< 0.005 (if pH 6.5)
Arsenic	0.2	0.050
Boron	< 0.1	No data
Cadmium	< 0.1	0.002–0.005 (depending on hardness)
Cobalt	0.1	No data
Chromium	< 0.1	0.002
Copper	0.1	0.002–0.005 (depending on hardness)
Iron	2,100	1.000
Magnesium	290	No data
Manganese	110	No data
Nickel	0.3	0.015–0.150 (depending on hardness)
Selenium	< 0.1	0.005
Zinc	5.7	0.005–0.050 (depending on hardness)
Calcium	500	No data
Potassium	< 1	No data
Molybdenum	< 0.1	No data
Sodium	220	No data
Phosphorus	< 1	No data
Sulfur	2,700	No data

Source: Harris and Ragusa (2000)

For example, the ANZECC has established guideline values for water quality criteria, which are well below the concentration levels of pollutants detected at Nairne Pyrite Mine, a known contamination site, as shown in Table 2. Wet chemical analysis was once used to monitor pollutant levels at contaminated sites. However, such traditional techniques have been replaced with ones that are significantly improved in chemical identification, accuracy, and in detection limits. A description of some of the analytical techniques that are currently used to analyze for organic and inorganic pollutants is presented in Table 3.

Various official methods are used to analyze for heavy metals in soils. Normally, these employ different acid mixtures and microwave heating systems, and mono- to multi-elemental spectroscopic techniques. The multi-elemental spectroscopic techniques, such as inductively coupled plasma-atomic emission spectrometry (ICP-AES), apply an optical atomic emission technique for performing sequential or simultaneous determination of a large number of analytes. Alternatively, the inductively coupled plasma-mass spectrometry (ICP-MS) method is dependent on ion counting instead of photon counting, and this approach has high sensitivity.

Analysis of organic pollutants in solid matrices involves drying, extraction, and detection steps, and there are various techniques available to accomplish these steps. The different methods used for sample drying and extraction include

Table 3 Instrumental or other methods used to analyze for selected organic and inorganic pollutants

Pollutant	Probable sources	Analytical methodology
Organochlorine pesticides	Insecticides	Gas chromatography (GC) High-pressure liquid chromatography (HPLC)
Polychlorinated biphenyls (PCBs)	Dielectric, heat transfer and hydraulic fluids, lubricants, plastics, adhesives, copying paper, etc.	GC/mass spectroscopy (MS)
Dioxin (2,3,7,8-tetrachloro-dibenzo-*p*-dioxin)	By-product in several chlorinated phenolic compounds; present in flyash, flue gas	GC/MS
Acrylamide-(2-propenamide or ethylene carboxamide)	Used as ground and soil stabilizer; adhesive, food packing, photography, oil recovery, metal coating	HPLC
Cadmium	Rock phosphate, alloys, batteries, pigments, plastics, electroplating, potteries, municipal wastes	Atomic absorption spectroscopy (AAS)
Mercury	Electrolytical processes, catalysts, dental amalgam, paper and pesticide manufacture	AAS (cold vapor technique)
Nickel	Nickel salts in geological materials, plumbing from industries	AAS
Nitrate and nitrite	Inorganic fertilizers, explosives, glass, heat storage systems, cured meat, printing	Nitrate is reduced and combined nitrites estimated by ion chromatography

the following: thermal, freeze-drying, or chemical drying followed by extraction by Soxhlet, sonication, KOH digestion, mechanical shaking, supercritical fluid extraction, accelerated solvent extraction, or microwave-assisted extraction. Various detection techniques are employed. Those that are most common include high-resolution gas chromatography–mass spectrometry (GC-MS) and liquid chromatography (LC)–fluorescence. However, there are many emerging pollutants and many among them are not amenable to simple and quick analysis. Lack of suitable instrumental analytical techniques often limits the capacity to identify the chemical classes and/or individual chemical components in mixtures that have a diverse organic and inorganic pollutant profile. Lack of such methods or techniques may contribute indirectly to the neglect of certain chemical substances that are environmental contaminants.

The advancement of analytical techniques based on GC-MS, LC-MS, tandem mass spectrometry (GC-MS/MS and LC-MS/MS) has allowed researchers to identify and measure a broad range of environmental chemical contaminants. Additionally, the application of time-of-flight MS (TOF-MS) has allowed science to accurately confirm the identity of analytes from complex matrices, thereby avoiding false-positive detections. The ability of TOF-MS to provide an accurate mass of the parent pollutant and its degradation product is of great advantage when studying the environmental behavior of mixed contaminants. Conversely, the commonly used separation methods, based on ICP (inductively coupled plasma) spectrometry, which are applied to the analysis of heavy metals and metalloids, do not provide inherent information about the chemical species because of limitations. Such limitations include (a) the complexity of environmental samples that often interfere with separation processes and (b) lack of analytical standards that permit chemical identification via matching of retention times. Fortunately, these issues can be resolved by using hyphenated techniques such as LC-MS and LC-ICP-MS, since ICP and MS can provide information about the element itself, as well as on the intact molecular species. Speciation methods have also been developed that are based on LC-ICP-MS. This approach allows for environmental speciation of chromium (Cr(III) and Cr(VI)), selenium (Se(IV) and Se(VI)), bromate and bromide, and zinc–aminopolycarboxylic complexes using recently developed methods (Chen et al. 2007a, b, 2008, 2009). Nevertheless, the need for ever more sensitive and selective analytical techniques continues because accurate monitoring of increasingly low concentrations of mixed pollutants is required. Another important advancement that also requires continuous improvement is unattended continuous pollutant monitoring. Unattended monitoring is important particularly, when larger and longer duration–mixed pollutant monitoring programs are undertaken.

Most existing analytical procedures are concerned with only specific groups of compounds. Compared to water, analyzing solid media such as soil, sediment, and sludge poses a significantly greater challenge. Developing extraction methods, especially for the emerging contaminants such as perfluorinated chemicals, hormones, and other endocrine disrupting chemicals, is rendered difficult because of their ability to sequester and adsorb to solid matrices. Methods have been reported for determining perfluorinated carboxylic acids in sediment, sludge, dust, and soil using HPLC-MS/MS (Moriwaki et al. 2003; Dinglasan et al. 2004; Higgins et al. 2005; Wang et al. 2005). Recently, analytical methods have been developed that are based on LC-MS/MS for simultaneous determination of different classes of pollutants. Such methods are preferred because they provide for a deeper understanding of the fate and behavior of different chemicals in the environment. There are several examples in the literature in which the relatively sophisticated methods that have been developed to detect and quantify mixtures of pollutants are described. Castiglioni et al. (2005) developed a multiresidue analytical method utilizing a solid-phase extraction and HPLC-MS/MS detection to measure 30 compounds. Included among these 30 were the following agent types: antibiotics, anti-cancer drugs, anti-inflammatories, gastrointestinal drugs, estrogens, and lipid regulators. Holmgren et al. (2005) determined 21 chemical explosive residues using an LC-MS method. Rissato et al. (2005) developed a multiresidue method that was based on a GC-ECD screening for several pesticides, in which the following agent types could be analyzed: organohalogens, organonitrogens, organophosphorus compounds, and synthetic pyrethroid insecticides. Confirmation of the chemicals was performed by monitoring selected ions using GC-MS. Also, Park et al. (2010) developed a multiresidue analytical method for 62 pesticides in soils using GC-ECD. Lega et al. (1997) developed an analytical method for the simultaneous determination of OCPs, PCBs, PAHs, phthalates, chloroaromatics, phenolics, phenoxy acids, and other base/neutral compounds in sewage sludge. The interactive effects of inorganic and organic pollutants need more attention because such effects are most relevant to soil environments receiving mixture of organic and inorganic pollutants.

5 Interaction Effects of Pollutant Mixtures Toward Soil Microorganisms

Most studies on microbial pollutant effects are based on the "single chemical substance approach"; therefore, the premise is that chemical interactions at polluted sites that contain multiple chemical substances either do not occur or are not toxicologically important at low concentrations. Yang (1994) observed that nearly 95% of the papers published in toxicological journals dealt with a single chemical substance or pre-exposure to one chemical substance followed by exposure to another one. Knowledge of the effects of pollutant mixtures may be considered beneficial, neutral, or adverse, depending on the component pollutant, organism, and endpoint studied. There are methods of analysis to test *interaction* in different disciplines such as statistics, epidemiology, and toxicology. Using these methods, Bailar and Bailer (1999) drew different conclusions from the hypothetical data on a mixture of two chemical substances that had synergistic, independent, or antagonistic interactions. These differences have clear implications for risk assessment. Simple mixtures are often considered to constitute only a combination of individual chemical substances that interact in an additive manner. Toxicity greater than additive is known as a synergistic effect, whereas toxicity lower than the additive effect is referred to as an antagonistic effect. In many cases, the toxicity data for simple or complex mixtures are not available (Monosson 2005).

Pollutant mixtures vary in the effects they have on microorganisms; they can be innocuous, additive, synergistic, or inhibitory. Certain terms are used to describe the effects of mixtures. Those mixtures that have "no interaction action" or "a zero interaction between components" on organisms conform to the basic concepts of "Loewe additivity" (Loewe and Muischnek 1926) and "Bliss independence" or the response additivity (Bliss 1939). In the Loewe additivity concept (also called dose additivity, concentration additivity, or "toxic unit approach"), a toxicant is considered not to react with itself, and each toxicant in the mixture acts in the same way, by the same mechanisms and reaches the same biological target. Therefore, the components are also purported to act in a simple similar way. The Bliss independence is based on the assumption that the mode of action and possibly, but not necessarily, the nature and the site of the effect differ among the toxicants in the mixtures. According to the characteristics of Bliss independence, the components are presumed to act in a simple dissimilar way. In addition to their effects on microorganisms, the individual pollutants within a mixture can interact in several ways. Bliss (1939) also identified the potential for interactions of chemical mixtures and defined three categories of interaction or "joint action" of chemical substances as follows: (i) independent joint action, (ii) similar joint action, and (iii) synergistic action. Independent joint action refers to the effects of chemical substances that act independently and have different mechanisms. The presence of one chemical substance will not impact the toxicity of another, and the combined toxicity can be predicted from knowledge of the independent chemical substance in the independent joint action. Similar joint action refers to the effects of chemical substances that cause similar effects, often through similar mechanisms, and the presence of one chemical substance may affect the impact of another chemical substance. Synergistic action results "when the effect of the combination is greater than that suggested by the component toxic effects" (US EPA 2000). Thus, the synergistic effect is greater than additive toxic effects. When toxicity is lower than an additive effect, it is antagonistic or inhibitory (Konemann and Pieters 1996).

To assess risks from low-level exposures to chemical mixtures, additivity concepts are commonly used (US EPA 2000). The assessment methods that utilize additivity concepts include the following: (i) the *dose addition*, (ii) the *response addition,* and (iii) the *effect addition* method. The important assumption for the dose addition method is that the toxic mechanism of action is the same across the components of a mixture, while toxic action is independent across the components of a mixture for the response addition method. The effect of the mixture is estimated directly from the summed dose of the exposure levels of similar components (after scaling for relative potency among components) in the dose addition methods. In the response addition method, the summation of individual risks is done after estimating the probabilistic risk of an effect for each individual chemical substance in the mixture. The effect addition occurs when the expected effect of a mixture is the arithmetic sum of a measured toxic effect of the single chemical agent in the mixture. These approaches are useful when the toxicological dose–response information for all of the chemical substances in a mixture is unavailable.

The statistical analysis of chemical mixture toxicology data or environmental risk assessment is fraught with many difficulties due to different concepts of joint toxic action. Gennings et al. (2005) proposed a unifying concept to address the contribution to toxicity of a chemical substance in the presence of one or more components in a mixture; their concept was based on the slope of dose–response curve(s). The authors provided an algebraic equivalence between different statistical additivity models, and the definition of additivity was given by Berenbaum (1985). If the slope of the dose–response curve of one chemical substance does not change in the presence of others in a mixture, then there is no interaction between the first chemical and the others. If the rate of change in response to the dose of the first chemical is altered in the presence of other chemicals, then an interaction is said to exist. Such generalized approaches for describing additivity or lack thereof in chemical mixtures are strongly needed, particularly if they place emphasis on low-dose regions (Teuschler et al. 2002). Olmstead and LeBlanc (2005) proposed a heuristic model that relies on addition and independent joint action to predict the toxicity of a chemical mixture. The heuristic model is based largely on the actions of a single constituent of the mixture that dominates toxicity, but without considering the possible interactions between the chemical substances. Most individual chemicals have no or low toxicity. But chemical mixtures may become toxic from the synergistic or antagonistic interactions among constituents. Hence, the information on the known toxicodynamics of the chemical substances involved, as well as the actual analysis on interactions, is essential to identify and corroborate the interactions that exist among the chemical substances. Finney (1942) used coefficients of interaction to describe the modifying effects of a single chemical on another.

The way microbes respond to a mixture of pollutants depends on the effects of the individual pollutants as well as those of the mixture at the individual, population, or community level. The nature of the chemical substance(s) and the duration of exposure determine the direct acute toxic action (lethality or reduced multiplication) or sublethal (physiological stress) effect (Long et al. 1995). Indirect toxicity results largely from pollutant effects on tolerant species and may occur through several ecological mechanisms; such effects may be manifested at the population, community, or ecosystem level (Fleeger et al. 2003). Interactions among chemical pollutant(s) and microorganism(s) can be simple to complex. While simple organic substances are effectively utilized by microorganisms for growth and energy, dealing with the complexity of organic and inorganic mixtures may require the involvement of entire microbial communities. In nature, microorganisms do not live on their own, but in large communities. The *complexity–interaction pyramid* gets wider as depicted in Fig. 2, when there are more individual pollutants in a mixture or more microbial species in a community are involved in interacting with the mixture. The bioavailability of pollutants with which the microorganisms come into contact depends

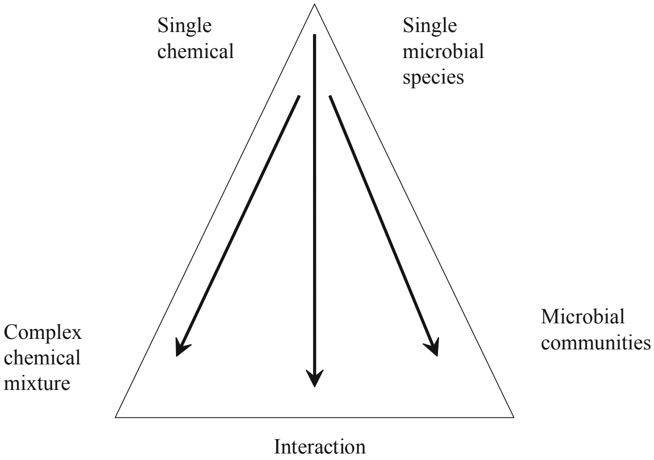

Fig. 2 Complexity–interaction pyramid between chemical pollutants and microorganisms

on the particular species and their relative abundance within the microbial community. Biosorption from complexation with cell wall polysaccharides, along with intracellular uptake, is an important factor that affects bioavailability of metallic contaminants (Ford and Mitchell 1992). Generally, the bioavailability of pollutants is influenced in the following ways: (i) chemical and physico-chemical interactions with other constituents of soil or water, (ii) physiological activities that modulate the levels of pollutants available for uptake, e.g., factors affecting uptake from the soil solution or water, and (iii) interactions among the microbes themselves or those between microbes and other organisms (Van Gestel and Hensbergen 1997).

Many currently available models that address mixed-substrate kinetics use simplified assumptions concerning how microbes behave. For example, they assume that (i) competition for growth substrates is the only interaction that occurs within the microbial population, which is often considered to be a single biomass and (ii) all chemical species together are considered to account for the BOD. When individual chemical species are considered, the assumption is that there is either no interaction or that the nature of the interaction is competitive. Reardon et al. (2002) developed a model entitled "sum kinetics with interaction parameters" (SKIP) using *Pseudomonas putida* F1 and *Burkholderia* sp. strain JS150. The authors grew these organisms individually and together on benzene, toluene, phenol, as well as mixtures of these solvents. The interactions between species in the mixed-culture experiments were substrate dependent and could not be predicted from simple competitive enzyme models. The SKIP model used parameters that were different from single- and dual-substrate mixture experiments to accurately predict the outcome of the three-substrate mixtures. The results of most studies usually provide data only on the toxic effects of parent compounds but ignore the degradation products that also may be inhibitory. The additive, synergistic, or antagonistic response of phototrophic microorganisms to atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine), a commonly used herbicide, and its main degradation products, viz., deethylatrazine (2-chloro-4-amino-6-isopropylamino-1,3,5-triazine) and deisopropyl-atrazine (2-chloro-4-ethylamino-6-amino-1,3,5-triazine) was reported by Stratton (1984). When degradation of one component is inhibited by another, the occurrence of contaminants in a mixture becomes very critical.

6 Nontarget Effects of Pollutant Mixtures

The environment generally contains a complex array of chemical substances that are derived from various origins. Such chemical substances usually have differences in their energetic values (Table 4). The pollutant-degrading microorganisms, like other heterotrophic microorganisms, derive energy that is required for essential functions such as the oxidation of chemical substances. The minimum energy quantum, i.e., the critical ΔG value, which can be harnessed to support microbial metabolism, is equal to the amount of energy required for the synthesis of one-fourth to one-third of one mole of ATP ($\sim$15–20 kJ mol^{-1}). The chemical substances that act as substrates for growth of microorganisms in soils are nevertheless scarce, because most

Table 4 Energetic values of selected compounds of biological interest in the environment

Compound	Formula	Molecular weight	kJ mol^{-1}
Water	H_2O	18	−237.2
Carbon dioxide	CO_2	44	−394.4
Oxygen	O_2	16	0
Hydrogen	H_2	2	0
Glucose	$C_6H_{12}O_6$	180	−917.3
Acetate	$C_2H_3O_2$	59	−369.4
Methane	CH_4	16	−50.8
Methanol	CH_3OH	32	−175.4
Nitrous oxide	N_2O	44	+104.2
Ammonium	NH_4^+	18	−79.4
Cellulose	$n(C_6H_{12}O_6)$	>400, 000	–
Hemicellulose	$n(C_5H_{10}O_6)$	–	
Fats	$C_{57}H_{110}O_6(l)$	890	−75520

Adapted from Dilly (2005)

carbon substrates (0.8–2%) are present as recalcitrant humus. Freshwater environments are estimated to have 6–10 μg carbon L^{-1}, most of which is unavailable for microbial growth. In the environment, the mixtures of chemical substances seldom stay in the form of original composition and they are acted upon photochemically and biologically. Many organic chemicals that are introduced into soil are eventually metabolized by microorganisms, because these organisms possess extremely active metabolic capabilities. Many inorganic substances are also microbially transformed, either by oxidation or reduction. Knowledge of the amounts of energy generated or required for degradation or transformation of chemical substances is critical to an understanding of microbial metabolism of pollutants. Most pollutants are environmentally stable and are normally present at low concentrations. The microbial metabolism of many pollutants, in which they are metabolized and perhaps detoxified, results from incidental metabolic activity, wherein microorganisms do not utilize them as an energy source (Matsumura 1987).

Microorganisms are seldom exposed to single chemical substances in the environment. The microbial metabolism of a chemical in a mixture is often influenced by other components of the mixture (Klecka and Maier 1988; Egli 1995). Depending on the microbial metabolic role each chemical component of a mixture plays within the mixtures, Harder and Dijkhuizen (1982) proposed the terms "homologous" and "heterologous" to describe those substances that serve the same or different roles, respectively. Increased microbial growth may occur, because biodegradation of a chemical substance generally occurs in the presence of other chemical components; this usually occurs at low substrate concentrations and in a mixture of homologous carbon and energy substrates (McCarty et al. 1984; Schmidt and Alexander 1985). In contrast, competitive inhibition, toxicity, and the formation of toxic intermediates by nonspecific enzymes can lead to inhibitory effects on growth and activities of microorganisms. The microbial metabolism of an individual pollutant may produce many reaction products that result from a multitude of enzymatic reactions. The fate of chemicals in the environment depends on what the rate-limiting reaction is for a given compound and what stable toxic metabolite(s) is (are) formed. In chemical mixtures, wherein no component inhibits or stimulates the microorganisms, labile components may be degraded first, leaving more stable components.

As biodegradation proceeds, the interactions that occur among microbial species are as important as the interactions that take place among components of a chemical. The diversity of ecological relationships among microorganisms, in terms of competition, cooperation, etc., is more important than factors such as phylogenetic diversity (Kawanabe 1996). Synergism and antagonism thus play a critical role in what the relative microbial population abundances are in the face of environmental stresses such as pollution (Atlas 1984). The mere inhibitory effects of chemical substances on microbial cells do not provide insight as to why those cells are inhibited. Recently, Brown et al. (2008) used the genomic array technology to investigate the effects of a nitroaromatic compound, *p*-nitrophenol (PNP), on *Escherichia coli* K12-MG1655. From his research, he was able to describe the global changes that occurred in gene expression. The exposure to PNP caused *E. coli* cells to prematurely enter a stationary phase, as shown by downregulation of the genes involved in protein synthesis (*rpl, rps, rpm*) or upregulation of the genes of *emrRAB* operon. These genes confer resistance to compounds that uncouple oxidative phosphorylation and induce the *marRAB* operon and *dps* gene, which bestows oxidative stress resistance. A downregulation of genes that code for porins, which transport solutes in the outer membrane of cells (*ompA*, *ompC*, *ompF*, and *ompT*), indicated that PNP also affected cell membrane constituents. The gene *rpoE*, which encodes a sigma factor that is involved in the response of cell envelope stress, was unregulated. The structurally similar dinitrophenol (DNP), a protonophore that uncouples oxidative phosphorylation, was also found to induce the *marRAB* operon. Toxicity data from a single species cannot be used to predict safe levels of a substance for ecosystems because of the complexity of their structure and function.

The exposure to toxic metals, either short term or long term, can reduce diversity and activities of soil microorganisms (McGrath et al. 2001; Lasat 2002; Hafez and Elbestawy 2009). The relative toxicity or bioavailability of a metal/metalloid pollutant generally depends on its chemical speciation (De Haan and Visser-Reyneveld 2004). After an initial inhibition by higher concentrations of metal pollutants, microbial activities and functions may recover because (i) there is a gradual decrease in their availability by immobilization reactions in soils and (ii) tolerant microorganisms may develop (Holtan-Hartwig et al. 2002). Such insights often come from the short-term studies limited to a single soil type and are conducted under controlled laboratory conditions. Hence, not all reports available on the toxic effects of individual heavy metal on soil microorganisms and their activities are in agreement (Vig et al. 2003). The results of laboratory studies are often difficult to extrapolate meaningfully to the effects of pollutant(s) that occur under field conditions. Giller et al. (1998), who reviewed the toxicity of heavy metals to microorganisms and microbial processes in agricultural soils, suggested that long-term field experiments are essential to understand how the gradual increase in the concentration of metal(s) in soils affects microorganisms. In a long-term contaminated agricultural soil that was adjacent to an industrial area, there was a significant decline in the genetic diversity of soil microorganisms (Hafez and Elbestawy 2009).

Microbes commonly develop tolerance toward heavy metals (Baath 1989; Gadd 1992). This response is attributed to long-term genetic modifications, the spread of resistance genes (through plasmids) among organisms, or replacement of metal-sensitive strains by tolerant ones. The time required to develop such community tolerance can be highly variable. Diaz-Ravina and Baath (1996) detected an increased tolerance in soil bacterial communities after 2 days of metal exposure, while Doelman and Haanstra (1979) found that it took 3 years to develop. The recovery in the activity of bacteria (thymidine incorporation rate) or fungi (acetate-in-ergosterol incorporation rate) and growth rates, after heavy metal introduction, does not necessarily imply the development of tolerance. It may derive from a gradual immobilization of metals by the soil matrix. In most ecosystems, the microorganisms that metabolize pollutants also utilize many natural substances at low concentrations. Certainly, these substances and other environmental factors, such as light and the presence or the absence of oxygen, influence the fate of organic pollutants of different classes (Table 5).

Table 5 Fate of organic pollutants as influenced by environmental factors

Chemical pollutant	Environmental factors	Possible process involved
Chemicals with chromophoric functional groups	Light, photoquenching photoactivation agents	Abiotic photodecomposition
Chemicals with high water solubility	Diffusion coefficient, matric-potential gradients	Transport by mobility in water
Chemicals with high Henry's Law constant	Concentration in air and water	Transport by volatilization
Chemicals with elevated octanol:water partitioning coefficients	Abundance and physiological capacities of organisms	Bioaccumulation
Chemicals with electron-dense center atoms	Presence of oxygen	Monooxygenase- and dioxygenase-based reaction
Chemicals with phenolic- and aniline-based structures	Oxygen and humic substances	Phenoloxidase- and peroxidase-based reaction
Chemicals with electron-deficient center atoms	Anoxic condition Nucleophile concentration (sulfide, amines, etc.)	Reductase-based reaction (reductive dehalogenation)
Chemicals with nucleophilic substitution	Water content, temperature, pH	Hydrolase-based reaction

Sources: Schwarzenbach et al. (1993) and Lyman et al. (1990)

6.1 Microbial Population Dynamics

Microbial growth and metabolism describe physiological processes at two different levels of complexity. In Fig. 3, we depict the energy flows that occur in a microbial cell leading to metabolism and cell synthesis. Catabolic or anabolic activities

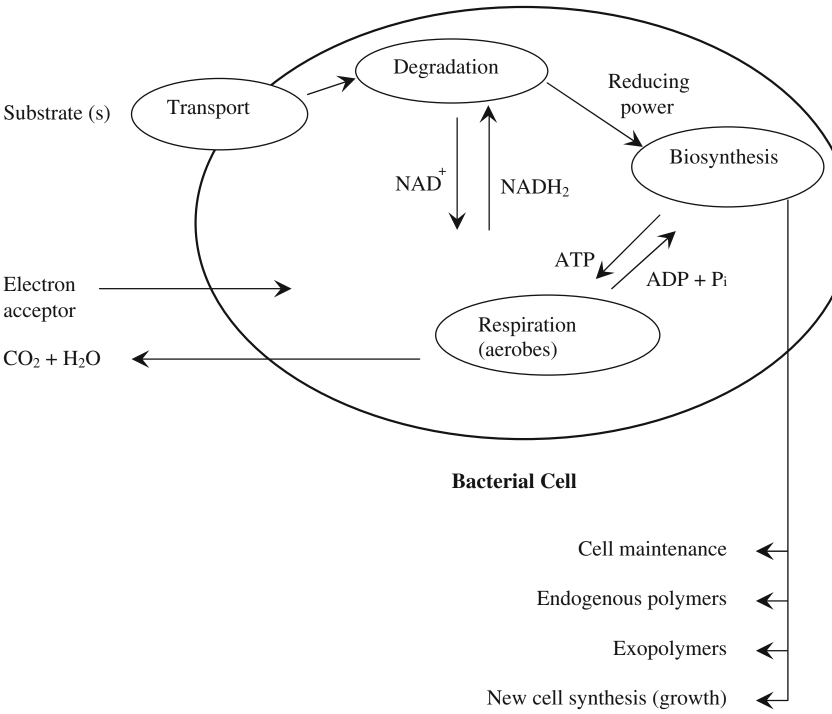

Fig. 3 Diagrammatic of energy flow in a metabolizing microbial cell. Adapted from Brock (1987)

describe the array of chemical reactions that ultimately generate energy and are responsible for cell maintenance, transport, biosynthesis, synthesis of exopolymers, and motion. Microbial growth results from anabolic reactions that allow cells to divide and increase in number. Certain physiological activities of microbial groups have economic importance for use in bioleaching, bioprecipitation, biodegradation, and biotransformation of pollutants as presented in Fig. 4. It is traditional to perform an ecological risk assessment at a polluted site to monitor the alterations that occur in microbial populations by the plate counts on unamended (control) and pollutant-amended growth media (Thompson et al. 1999; Ellis et al. 2001).

Soil microorganisms are more sensitive to metal pollution than are meso- or macro-fauna or flora (Giller et al. 1998). Intracellular concentrations of metal ions are highly regulated, and several specific resistance mechanisms contribute to cellular metal homeostasis (Table 6). Heavy metals can cause shifts in the composition of soil microbial populations (Griffiths et al. 1997) and detrimental effects on microbial cell metabolism at high concentrations (Petersen et al. 2003). Mitchell et al. (1978) showed that total and viable bacterial populations were higher in sludge-treated soils than in arable soils, but this effect declined with time. Slow mineralization of organic matter in sludge could release more soluble forms of the metals (McBride 1995). Otherwise, the bioavailability of trace metals in sludge is very poor (Shuman 1999);

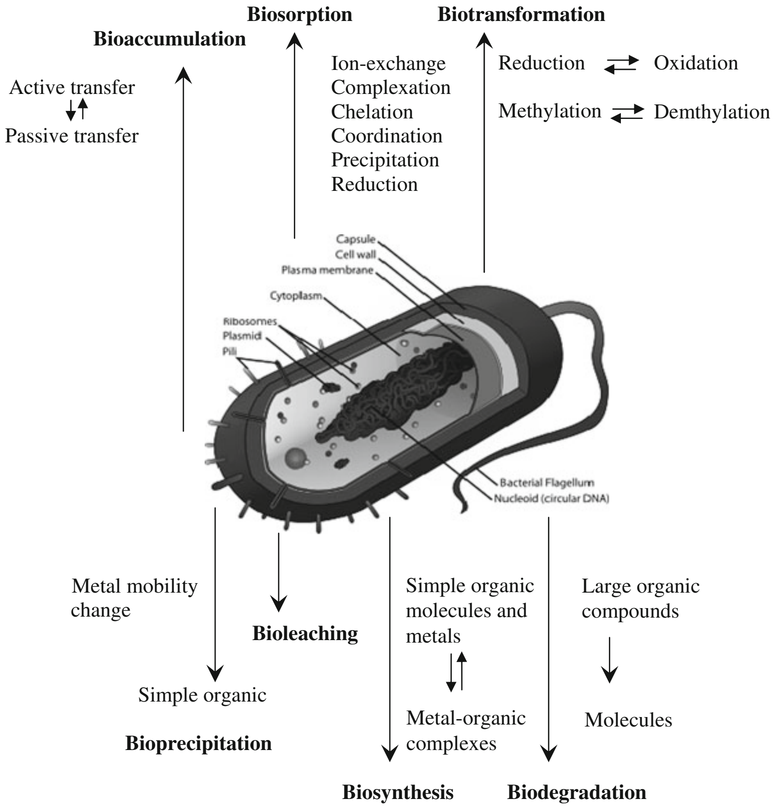

Fig. 4 Physiological activities of a bacterium that form the basis for different strategies of pollutant treatment

the sludge-borne metals mainly exist in reactive forms on the surfaces of soil particles (McGrath and Cegarra 1992). Chang et al (1997) proposed the "sludge time bomb hypothesis" to describe the effect, wherein more soluble forms of metals are released from sludge. Many different metals may be present in sludge and the synergistic or antagonistic interactions of those constituent metals determine the toxicity to the organisms present. For example, Zn and Cd may produce antagonistic or synergistic effects depending on their respective concentrations and the nature of the response in a target organism (Adriano 2001). Certain groups of microorganisms are more sensitive to metal pollution than are others. Fungi are more resistant than are culturable heterotrophic bacteria to heavy metal pollution (Baath 1989). Oliveira and Pampulha (2006) observed a marked decrease in the number of asymbiotic nitrogen fixers and heterotrophic bacteria in soils contaminated with mercury (109 mg kg^{-1}) and arsenic (1,558 mg kg^{-1}) for more than 40 years.

A few organic chemical substances are capable of killing diverse types of organisms and are known as biocides. Dean (1978) showed that phenols inhibit the

Table 6 Mechanisms of metal resistance in microorganisms

Mechanism of resistance	Metal	Selected reference
Enzymatic detoxification	Hg^{2+}/organomercurials	Misra (1992), Osborn et al. (1997), and Miller (2007)
Transport out of the cell	Hg^{2+}	Begley et al. (1986), and Siciliano and Lean (2002)
Reduction reaction	AsO_4^{3-}/AsO_2^{-}	Newman et al. (1998), and Zhang et al. (2008b)
Involvement of ATPase in pumping out of cell	AsO_4^{3-}/AsO_2^{-}	Rosen et al. (1995)
Metal-specific efflux	Cd, Co, Cr, Cu, Hg, Ni, Zn	Nies (1995), and Nies and Silver (1995)
Pumping out of cells by an H^+ antiporter	Cd, Co, Cr, Cu	Sliver and Ji (1994)
Formation of volatile metal forms	Cd, Hg, Pb, Sn, Se, As	Summers and Silver (1978), and Lovely (2000)
Protein–metal chelation	Cd, Cu, Ni, Zn	Gadd and Griffiths (1978), and Higham et al. (1986)
Carbohydrate–metal chelation	Cu, Mn	Kidambi et al. (1995)

enzyme responsible for the oxidation of aromatic compounds, or adversely affect cell viability. Welp and Brummer (1992) found pentachlorophenol (PCP) to be the most toxic organic pollutant, followed by phenol and linear alkyl benzenesulfonate (LAS), when different organic compounds were tested for their toxicity to the microbial reduction of Fe(III) oxides to soluble Fe(II). Most organic compounds that are water insoluble, and are adsorbed and concentrated in sewage sludge, produce effects on soil microflora (Scheunert et al. 1995). Chaudri et al. (1996) observed that PCP at 200 mg kg^{-1} in soil decreased the indigenous population of *Rhizobium leguminosarum* biovar *trifolii*. Although laboratory study results showed increases in culturable bacteria and fungi after the addition of glyphosate, the long-term and repeated application of glyphosate produced minimal effects on seasonal microbial characteristics in the “Garden of Eden” experiment (Busse et al. 2001). As a polar compound, glyphosate is known for its strong adsorption to Fe and Al oxides and clay (McBride and Kung 1989). Its availability is unrestricted in artificial media, but binding to soil particles and metal complexes reduces the pool of labile glyphosate and, consequently, the uptake rate by soil microorganisms. In contrast to the scientific reports on the effects of heavy metal pollutants, information on the effects of organic pollutant mixtures on microbial population dynamics are limited.

The mixtures of organic pollutants can be catabolized to antagonistic products or may enhance the biodegradation of parent compounds by microorganisms (Shimp and Pfaender 1985). Verrhiest et al. (2002) observed that the PAH mixture (phenanthrene, fluoranthene, and benzo(k)fluoranthene) at 30 mg kg^{-1} (total concentration of three chemicals) had no effect on total bacteria or viable bacteria. However, the same mixture at 300 mg kg^{-1} decreased the quantity of total bacteria and the proportion of viable bacteria. Because of the complexity of the soil environment and of microbial interactions within and among different ecological groups, many techniques are required to reliably quantify the effects of pollutant mixture on microorganisms. The challenge is further compounded by the poor understanding of the seasonal population variations and the environmental parameters that control the variations among different ecological groups of microorganisms.

6.2 Microbial Biomass

Microbes are good bioindicators of pollution because of their central and critical roles in the soil ecosystem function. Monitoring of microbial dynamics is useful for detecting soil disturbances effected by pollution. The classical methods for enumerating microbes employ direct microscopic counting techniques or the statistical method of the "most probable number" technique. The reliance is more on microbial growth, and on the selection of culturable microorganisms. But these methods obliterate most of the interactions between microbial members and different components within the environment. The microbiological properties that can be easily and economically measured, and also sensitive and robust, would serve as reliable soil pollution monitoring indicators (Brookes 1995). Soil microbial biomass is used as one such indicator for soil quality and health in environmental monitoring. The microbial biomass is regarded to constitute the entire microbial community. Moreover, it is a source–sink for major nutrient elements such as N, P, and S as well as for C (Smith and Paul 1990). The microbial biomass contains 1–3% of the total soil carbon and approximately 5% total soil N. There are many reports on the effects of heavy metal pollutants on soil microbial biomass. The presence of combinations of metals had greater adverse effects on soil microbial biomass than those caused by single metals at high concentrations (Chander et al. 1995; Khan and Scullion 1999). The accumulation of heavy metals from sewage sludge disposal, when used as soil amendments on agricultural land, had adverse effects on soil microbial biomass (Brookes and McGrath 1984; Chander and Brookes 1991, 1993).

The relationships between the amount of soluble and exchangeable metals extracted by solutions of various electrolytes and chelating agents are less clear, as are the effects of more than one heavy metal on soil microorganisms. There are reports on the aftereffects on soil microbial biomass of past applications of sewage sludge (Brookes and McGrath 1984; McGrath et al. 1995). Li et al. (2006) demonstrated that heavy metal contamination decreased soil microbial biomass in samples of farmland that stood at different distances (0.01–5 km) from a copper and zinc smelter. Stratton and Steward (1992) found no change in microbial biomass in mineral soil and a stimulation of microbial biomass in litter following addition of glyphosate to an acidic forest soil. Kuperman and Carreiro (1997) suggested that the soils with higher organic matter content supported higher contamination as well as increased microbial biomass. In a study using soil samples from a creosote reservoir that was established about 50 years ago, Blakely et al. (2002) observed that fungal biomass was negatively associated with concentrations of pyrene and fluoranthene (which ranged from 2.04×10^{-5} to 6.46×10^{-4} $\mu g\ g^{-1}$ dry soil), whereas the bacterial biomass correlated positively with increased concentrations of PAH (reaching 10^8–10^9 μg g^{-1} in dry soil). At long-term total petroleum hydrocarbon (TPH)-contaminated sites, soil microbial biomass was found to be less in soils with medium to high level (5.2–21.4 g kg^{-1} soil) of TPH (Megharaj et al. 2000a).

Certain derived parameters, such as the microbial quotient (the ratio of microbial biomass-C to total soil organic carbon) and the metabolic quotient (the ratio of basal respiration to the total microbial biomass-C), provide information on the capability of a soil to support microbial growth and the efficiency by which the soil microorganisms use the carbon resources, respectively (Insam and Domsch 1988; Insam and Haselwandter 1989). The better quality soils are expected to have a higher microbial quotient, and stressed soils are expected to have a high metabolic quotient, compared to less-stressed soils. These parameters are also used to indicate whether the soil has vulnerability to disturbance in terms of resilience and resistance (Seybold et al. 1999). Both the soil resilience, which refers to its capacity to recover its functional and structural integrity after a disturbance, and the soil resistance, which refers to its capacity to continue to function without change throughout a disturbance, are related to soil quality through loss and recovery of soil functions.

The types and levels of nutrients in the microbial biomass (MB) are measured by using different conversion coefficients: MB-N (nitrogen) with $K_N = 0.54$ (Brookes et al. 1985); MB-P (phosphorus) with $K_P = 0.40$ (Brookes et al. 1982); and MB-S (sulfur) with $K_S = 0.35$ (Chapman 1987). Vasquez-Murrieta et al. (2006) reported a significant negative correlation ($P < 0.0001$) between microbial biomass, soil organic carbon, total N and carbon (C) mineralization, and heavy metal content (As, Pb, Cu, and Zn) in a long-term contaminated soil near a mine in San Luis Potosi, Mexico. From studies that used soil contaminated with Pb, Zn, Cu, and Cd from a copper mine in Zhejiang Province, China, Liao and Xie (2007) showed that microbial ecophysiological parameters, such as the microbial biomass C/microbial biomass N ratio and the metabolic quotient (qCO_2), were significantly correlated with heavy metal stress. In contrast, in soils contaminated over a wide area by sewage sludge, exhaust dust deposition of a lead factory and river sediments of mining residues, microbial biomass C, N, and P did not show any clear heavy metal effect that related to soil dry weight. But the ratios of microbial biomass C/soil organic C, biomass N/total N, and biomass P/total P were all negatively affected by increasing concentrations of Zn, Pb, and Cu, as detected by a source-specific analysis of covariance using the different heavy metal fractions as covariates (Khan et al. 2007).

There are also reports which suggest that microbial biomass is inadequate as an indicator of pollution. Renella et al. (2008) found that microbial species richness was increased only in soils from an As-contaminated site that were amended with C alone, or with compost alone, and in combination with beringite or zerovalent iron grit. These amendments were added to increase organic matter content and to reduce trace element mobility so that the growth of *Holcus lanatus* and *Pinus pinaster* would be enhanced. The microbial endpoints tested in this study were microbial biomass and activity, and microbial and plant species richness. According to Renella et al. (2008), these endpoints were not choice indicators of soil remediation practices. Several factors restrict the utility of soil microbial biomass measurements as an index to pollution. Those factors include (i) the lack of benchmarking values, (ii) too few data on noncontaminated background soils, and (iii) almost no data on the seasonal effects of microbial biomass in different soils (Conrad et al. 1999). In addition to the effects of a pollutant's dose that is applied, residue type, soil texture, and the confounding effect of soil organic matter limit our understanding of the relationships that exist between soil microbial biomass and soil pollution (Hofman et al. 2004; Kaschuk et al. 2010).

6.3 Effects on Microalgae and Cyanobacteria

Algae play a major role in the accumulation of hydrophobic pollutants. Exposure to PCBs at concentrations of 1–10 ppb may result in significant reductions in marine and freshwater phytoplankton size and biomass (O'Connors et al. 1978). Besides reductions in size and biomass, reduced photosynthesis, cell division, and growth have been documented to occur in response to increased PCB concentrations (Harding 1976; Harding and Phillips 1978). Paterson et al. (2003) studied the long-term response of diatom and chrysophyte communities in pollution-impacted and reference lakes (Saglek-2, SK-2), which had received PCB inputs in northern Labrador. Using sediment samples that were deposited during the past 150 years, they found that the chrysophyte and diatom assemblages were characteristic of clear, slightly acidic, oligotrophic lakes. There was little change in either community, suggesting that elevated PCB concentrations in lake sediments did not reflect the bioavailable concentrations present in lake water.

Algae possess acid phosphatase activity, which determines the availability and recycling of inorganic phosphate and autophagic digestive processes. The chemical substances released into the environment from agricultural activities may impair algal acid phosphatase activity. Jonsson and Aoyama (2007) evaluated the in vitro effect of 24 organic compounds and 6 metals, either used as pesticides or present as contaminants in sewage sludge on the acid phosphatase activity extracted from *Pseudokirchneriella subcapitata*. Only the LAS and the heavy metals Hg^{2+}, Al^{3+}, and Cu^{2+} markedly altered (about 50%) the enzyme activity. From joint action inhibition studies, the authors found that Hg^{2+} was a more potent inhibitor of acid phosphatase activity than was Al^{3+} or LAS, and that the $Hg^{2+} + Al^{3+}$ and Hg^{2+} + LAS mixtures had additive and slight antagonistic effects, respectively. When preincubated with the enzyme, copper showed an activation effect as well as a slight antagonist effect for Hg^{2+}. Kotrikla et al. (1999) observed that the presence of a mixture of atrazine and metolachlor in individually toxic concentrations lower than the EC_{50} resulted in reduced toxicity (antagonism), when compared with the toxicity caused by the sum of toxic actions of the same levels of single chemical substance on *Chlorella fusca* var *fusca*.

At contaminated sites, a parent chemical in combination with its degradation products can exert higher toxicity than does the parent chemical alone. For instance, Megharaj et al. (2000b) studied the impact of long-term DDT pollution in soil by using different criteria. The criteria used included chemical analysis of DDT residues [DDT, DDE (1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene) and DDD (1,1-dichloro-2,2-bis(4-chlorophenyl)ethane)], microbial biomass, dehydrogenase activity, viable counts of bacteria and fungi, and density and diversity of algae. DDT was the major contaminant followed by its degradation products, DDE (19–27% of total DDT residues) and DDD (3–13% of total DDT residues) present in low, medium, and highly contaminated soils. Low-level contaminated soils had minor quantities of DDD compared to medium- and high-level contaminated soils. Viable counts of microalgae and bacteria decreased with increasing DDT contamination, while fungi, microbial biomass, and dehydrogenase activity increased in the medium-level contaminated soil (27 mg DDT residues kg^{-1} soil). All of the tested parameters were greatly inhibited in the high-level contaminated soil (34 mg DDT residues kg^{-1} soil). The predominant algae present in the uncontaminated soil belonged to two species of Chlorophyceae (*Chlorococcum* sp. and *Chlorella* sp.) and two species of cyanobacteria (*Anabaena* sp. and *Nostoc* sp.). In the highly contaminated soil, only one species of microalgae was present, namely *Chlorococcum* sp., whereas in low- and medium-level contaminated soils, two species of algae were present, i.e., *Chlorococcum* sp. and *Chlorella* sp. Cyanobacteria were not detected in the contaminated soils. Thus, the species composition of algae and cyanobacteria was altered in contaminated soils, and sensitive species were eliminated in the medium and highly contaminated soils. Notably, a greater toxicity was observed in the medium and highly polluted soils, wherein DDD was present as a significant portion of the total DDT residues, suggesting an additive interaction among the DDT residues. Given the fact that DDD had a broader antimicrobial spectrum and was more toxic to microorganisms than were DDT and DDE (Megharaj et al. 1999), the observed greater toxicity in the medium- and high-level contaminated soils can be attributed to the presence of DDD.

Petroleum comprises a mixture of several aliphatic and aromatic hydrocarbons, and is a common contaminant of environmental concern. Although a wealth of information exists on the biodegradation of petroleum hydrocarbons, information on their toxicity to microorganisms in soil, particularly soil algae and cyanobacteria, is very limited. Megharaj et al. (2000a) reported on the toxicity of TPHs to soil algal populations, microbial biomass, and soil enzymes (dehydrogenase and urease) in a long-term TPH-contaminated site. Microbial biomass, soil enzyme activity, and microalgae decreased in medium- to high-level (5.2–21.4 g kg^{-1} soil) TPH-contaminated soils, whereas low-level (2.1 g kg^{-1} soil) contamination stimulated the algal populations and had no effect on enzymes and microbial biomass. However, more severe toxicity (as measured for all test parameters) was observed in the medium-level contaminated soil than in highly contaminated soil. This result is counter-institutive and could not be explained by chemical analysis alone. More importantly, an alteration in the species composition of algae was observed in contaminated soils as a result of the elimination of the sensitive species in the medium-to-high TPH-contaminated soils. The aqueous elutriates, prepared from those contaminated soils, inhibited algal growth, which also supported these results. Alterations in the composition of algal species proved to be more sensitive than microbial biomass or soil enzyme activity measurements. For adequate toxicological assessments, chemical analyses are inadequate by themselves and must be performed in conjunction with bioassays.

Algal systems can be effectively employed to remove chemical pollutants. The biological systems that are proposed for use in removing metal ions from polluted waters include the following: high-rate algal ponds (Oswald 1988), the suspended biomass of common green algae (Toumi et al. 2000), or immobilized eukaryotic microalgae/prokaryotic photosynthetic cyanobacteria (de-Bashan and Bashan 2010). The photosynthetic oxygen production by algae reduces the need for external aeration and this offers advantages for treating hazardous pollutants that must be biodegraded aerobically, but might otherwise volatilize during mechanical aeration.

Microalgae can be used to produce the O_2 required by populations of acclimatized bacteria that biodegrade hazardous pollutants such as PAHs, phenolics, and organic solvents (Muñoz and Guieysse 2006). Using a one-stage treatment involving an alga and bacteria, Borde et al. (2003) reported the photosynthesis-enhanced biodegradation of toxic aromatic pollutants such as salicylate, phenol, and phenanthrene. The presence of green alga *Chlorella sorokiniana* was synergistic for the biodegradation of salicylate by *Ralstonia basilensis*, phenol by *Acinetobacter haemolyticus,* and phenanthrene by *Sphingomonas yanoikuyae.* In the standard algal growth inhibition test for pollutants/contaminants, the microalga *Selenastrum capricornutum* is used. Gudia et al. (2008) evaluated the toxicity of a complex mixture of the fungicide "cymoxanil" (2-cyano-*N*(ethylamino) carbonyl-2-methox-amino acetamide) along with its many components and copper oxychloride (Cu-OCl), using a bioassay battery that included *Artemia franciscana*, *Daphnia magna*, and *S. capricornutum.* The endpoints measured were immobilization and cell growth inhibition. The toxicity of cymoxanil followed the following order: *S. capricornutum* $\cong$ *D. magna* > *A. franciscana*, while Cu-OCl exerted its toxicity in the following order: *S. capricornutum* > *D. magna* $\cong$ *A. franciscana*. The technical grade R6 (labeled as mixture of cymoxanil at 4%, Cu-OCl at 69%, and formulation ingredients at 27% of the total weight) displayed its toxicity as follows: *S. capricornutum* > *D. magna* > *A. franciscana*. According to Gudia et al. (2008), more species living in different environmental matrices such as water, soil, and sediment are to be included in future toxicity tests to obtain reliable data for appropriate risk analysis. In a recent study, Pokrovsky et al. (2008) described the surface properties of *Gloeocapsa* sp. and determined the identity and concentration of the major surface functional groups present. These included carboxylate, amine, phosphoryl/phosphodiester, and the hydroxyl responsible for the amphoteric behavior of cyanobacterial cell surfaces that adsorb metals. The information on the most efficient range of pH, cell biomass, and duration of exposure necessary for controlled metal adsorption on cyanobacterial cultures would allow optimization of metal bioremediation/biosequestration processes in metal–cyanobacterial cell interactions.

The few reports that are available on trace element mixtures show that they may have additive, synergistic, and/or antagonistic effects on algae (Khalil et al. 1996; van Gestel and Hensbergen 1997; Sharma et al. 1999). Walter et al. (2002) studied the combined effect of multiple mixtures composed of structurally dissimilar priority pollutants (atrazine, biphenyl, chloral hydrate, 2,4,5-trichlorophenol, fluoranthene, lindane, naphthalene, parathion, phoxim, tributylin chloride, and triphenyltin chloride) and statistically estimated the individual no-observed-effect concentrations (NOECs) using an algal (*Scenedesmus vacuolatus* Shih. et Krauss strain 211-25) biotest. The toxicity of the mixture was higher than expected for any single substance alone but was predictable using the concept of independent action (Fig. 5). Such effects can be demonstrated easily under laboratory conditions by adding known concentrations of pollutants singly and then in combination. But in a complex ecosystem in which soil may be contaminated by mixtures of pollutants, the overall impact (combined effect) on biological functions cannot be easily determined. Moreover, the contribution of individual pollutants and interaction among the mixed pollutants in a complex ecosystem is difficult to assess. Norwood et al. (2003) did a comprehensive review of the effects of metal mixtures on aquatic biota. The effects they recorded were more than additive in 30% of the cases, were less than additive in 43% of the time, and were strictly additive in 27% of cases. Such variability in effects was attributed to variables in the organisms exposed and numbers, types, and concentrations of metals tested. Moreover, the consequences of simultaneous interactions between two or more pollutants in the same alga remain poorly understood. Very recently, Ramakrishnan et al. (2010) therefore stressed the need to further investigate the impact of mixtures of environmental pollutants toward microalgae and cyanobacteria.

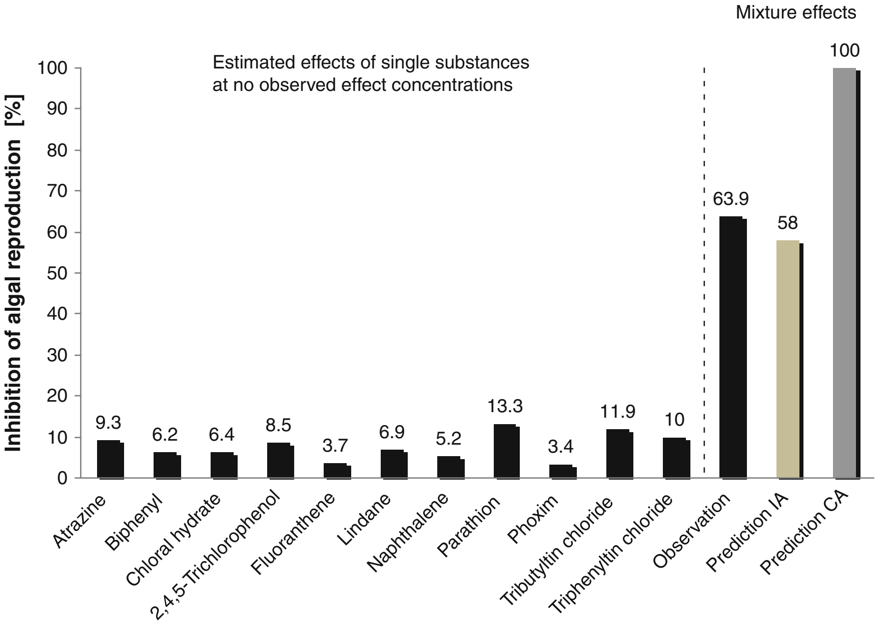

Fig. 5 Effect of structurally dissimilar priority pollutants, singly or as components in mixtures, on the reproduction of *S. vacuolatus*. Observed inhibition at individual no-observed-effect concentrations. Prediction IA, concept of independent action and prediction CA, concept of concentration addition. Walter et al. (2008), with permission from Kluwer Academic, the Netherlands

6.4 Microbial Community Structure

Microbial community structure is defined by the number of parts or elements within a system and by species richness and evenness. No single technique is currently available to describe an entire soil microbial community. Culture-based methods capture the nature of only a small fraction of the community. DNA-based profiling methods, such as denaturing gradient gel electrophoresis (DGGE) or terminal restriction fragment polymorphism (T-RFLP), depend on the selection of primers and polymerase chain reaction (PCR) conditions to describe the genetic structure of bacterial, archaeal, or fungal communities. But the difficulties associated with the extraction and quantification of microbial community DNA from soils accentuate biases in the DNA-based methods. The non-DNA-based methods, such as phospholipid fatty acid (PLFA) profiling, do not address the archaeal members. Despite the limitations of these methods, comparing microbial community structures is useful in pollution monitoring. Kozdroj and van Elsas (2001) have reviewed the methods employed for community structure analysis; some methods are based on signature biomarkers such as nucleic acids and fatty acids which can be used assess the impact of heavy metals and organic pollutants better.

Chemical pollutants can be a driving force that leads to the creation of dominant populations within perturbed microbial communities. Both enhanced physiological tolerances and substrate utilization capabilities can lead to a highly “specialized” surviving population. Generally, the taxonomic and genetic diversities of soil microbial communities that are disturbed by chemical pollutants were lower than in undisturbed communities (Mills and Wassel 1980; Atlas et al. 1991; Hafez and Elbestawy 2009). Almas et al. (2004) studied the acquired Zn and Cd tolerance of a soil microbial community and compared it with the chemical analysis of Zn and Cd in 10 soils that had differing pH characteristics, organic matter content, texture, vegetation/cultivation history, and metal contamination. They concluded that the total concentrations of Cd and Zn in extracted pore water (PW) appeared to be the best predictor of soil microbial exposure to trace metals. If the metal contamination of soils increased, the average number of aromatic acids utilized by bacterial isolates decreased (Wenderoth and Reber 1999). There can be a shift in the distribution of catabolic capabilities within metal-affected communities; increased stress alters the ratio between frequent and rare capabilities within microbial communities. Wenderoth and Reber (1999) segregated bacterial isolates by typing them with a twofold amplified ribosomal DNA restriction analysis (ARDRA) that employs the restriction enzymes *Alu*I and *Hae*III. They found that the bacterial isolates belonged to 12 distinct clusters, based on the analysis of the restriction patterns obtained with each of the two enzymes on the 100% similarity level. In contrast, Xia et al. (1995) demonstrated the absence of any effect of 2,4-D on different soil communities in an assessment of the molecular genetic response.

Hydrocarbon contamination selects for a less diverse but catabolically versatile bacterial community (Atlas et al. 1991). Sigler and Turco (2002) found nonsignificant changes in the community structure of dominant species, along with enhancement and inhibition of a variety of dominant organisms using DGGE analysis, following shortly after the single application of the fungicide chlorothalonil. Bundy et al. (2004) observed distinct shifts in microbial community structure, monitored using community-level physiological profiling (CLPP) and PLFA profiling, along with changes in the toxicity of the applied paraffin and motor oil to soil microcosms, using a luminescent bacteria test and the hydrocarbon degrader *P. putida* F1 (pUCD607) bioassay. Maila et al. (2006) studied the influence and relevance of the geographical location of the sample by artificially contaminating soils with different petroleum products. The particular hydrocarbon, rather than the geographical origin of the sample, was more important in determining functional or species diversity within the bacterial communities, when analyzed by the community profiling of both functional and molecular responses.

Soil microbial communities play a critical role in the recovery of soil at a disturbed site (Bending et al. 2000; Breure 2005). Perkiomaki et al. (2003) used a 2^2-factorial design with sulfuric acid (pH 3.1) and Cu–Ni addition to assess the effects of moderate amounts of continuous acid (acid and CuNi + acid) and metal (CuNi and CuNi + acid) deposition on humus microbial activity and community structure in the field, after nine growing seasons. They found that both acid and metal treatments changed the structure of the microbial community in the humus layer. The acid application decreased the humus layer pH and base saturation but increased the amounts of both extractable and bioavailable Cu. In addition, the metal application increased the concentration of extractable Ni and changed the fungal community structure, using soils that had been contaminated 25 years previously and still showed enhanced levels of either As or Cd (at 18 and 34 or 39 and 134 mg of As and Cd kg^{-1}, respectively). Lorenz et al. (2006) reported that bacterial community composition in contaminated soil differed from that in the control soil by the PCR-DGGE analysis; soil fungi and Proteobacteria appeared to be tolerant to As and Cd, while other groups of bacteria were reduced. In a study on the effects of combined application of mefenoxam, an acylanilide (*R*-isomer of metalaxyl) fungicide, and copper on the structure and function of soil microbial community, Demanou et al. (2006) found no significant effect on the mineralization rate of mefenoxam. Both copper and mefenoxam exerted selective detrimental effects on sensitive populations of fungi. Active bacterial populations were not adversely affected, but a new population structure could be observed during the exposure time. While copper resistance has been reported in many individual bacterial populations (Cooksey 1993), copper at 540 $\mu g\ g^{-1}$ had little effect on the bacterial community, when analyzed for community changes using amplified DNA fragments (Girvan et al. 2005). In a recent report, Hafez and Elbestawy (2009) observed a significant decline in the genetic diversity from clones analyzed using denaturing high-performance liquid chromatography, and partial sequencing for microbial 16S and 18S rDNA, in a Defsho soil and in an agricultural soil adjacent to an industrial drain in the Nile Delta that was highly polluted with heavy metals and PAHs.

Lorenz et al. (2006) used respiratory quinine analysis to assess the composition of a microbial community in long-term arsenic- and cadmium-contaminated soils. They reported that soil fungi and Proteobacteria were tolerant toward As and Cd, while other microbial groups were reduced in number. Vazquez et al. (2009)

used the T-RFLP technique, the most popular method to study microbial communities. They found temporal changes that occurred in a microbial community from a diesel oil-contaminated Antarctic soil by detecting *nahH* and *catA* genes involved in naphthalene and catechol biodegradation. Widenfalk et al. (2008) observed that there were significant shifts in the bacterial community analyzed by T-RFLP in soils exposed to glyphosate at environmentally relevant concentrations. Recent developments in molecular biology and bioinformatics provide new opportunities to gain a better understanding of the structure and function of microbial communities at polluted sites. Singh et al. (2006) introduced the multiplex T-RFLP for simultaneous profiling of multiple taxonomic groups of microorganisms. He et al. (2007) developed the GeoChip, the microbial ecological DNA microarrays to analyze phylogenetic or functional genes to characterize the microbial communities involved in environmental processes. The application of GeoChip would help to monitor the fate of pollutant mixtures efficiently. The de novo sequencing of microbial metagenomes, based on pyrosequencing, can facilitate the rapid generation of microbial community profiles (Hamady et al. 2008). McGrath et al. (2008) studied the mRNA transcriptional profiles to gain better understanding of the activities of environmental microbial communities. Further development of statistical algorithms and bioinformatics tools will improve the efficiency and promote the widespread application of many of these methods.

6.5 Functional Diversity of Microbial Communities

Individual species rely on the presence and interaction of other species. Therefore, community-level microbial functions are complex. The indicator for the functional diversity of a microbial community is generally based on metabolic profiling by the carbon utilization pattern (Nielsen et al. 2002). Zak et al. (1994) defined the functional diversity of a microbial community as the number, type, activity, and rate at which a suite of substrates is utilized. The carbon utilization pattern in CLPP suggests that the potential diversity in microbial communities is based on their ability to utilize a particular carbon substrate for growth (Garland and Mills 1991). Wunsche et al. (1995) observed clear shifts in the CLPP in soil contaminated with spindle oil, along with the development of a hydrocarbon-utilizing bacterial community. From their studies on the impact of long-term heavy metal contamination on soil communities, assessed by a number of methods, Ellis et al. (2001) suggested that CLPP and plate counts were useful for determining the gross effect of metals on soil microbial communities. The shifts in microbial community structure resulting from the additions of paraffin and motor oil could be monitored from the CLPP of the soil microcosms (Bundy et al. 2004). By using Biolog GN Microplates for CLPP, Avidano et al. (2005) found that the presence of chemical pollutants such as copper and mercury at one site, and 1,2,3,4-tetrachlorobenzene and 2,4-dichloroaniline, and arsenic and mercury at another site of a chemical factory in Cengio, Italy, led to more similarity in composition of the bacterial community, compared to that of the control site. Moreover, the chemical pollutant induced shifts in bacterial community structure by selecting out specific bacterial group that were better adapted to variability of pollutants. Zhang et al. (2008a) observed that microbial functional diversity, in terms of the CLPP patterns, increased with an increase in the amount of sewage irrigation. Since the CLPP patterns are biased toward the microbial members of only 3–4 genera (Ros et al. 2008), the CLPP method should be used only when combined with other techniques adequate for analyzing community structure.

6.6 Microbial Activities

Microbial activities refer to all the metabolic reactions and interactions among the microflora and microfauna that exist in soils (Nannipieri et al. 1990). Powlson et al. (1987) suggested that the abundance and activities of microorganisms reveal the degree of soil development. Heal and MacClean (1975) reported that about 90% of the energy in the soil environment flows through microorganisms. The commonly used methods for measuring microbial activities include rates of soil respiration, enzymatic activities related to the cycle of nutrient, and certain biogeochemical functions. Measures of microbial activities are considered to be good indicators of the degree of soil pollution (Tabatabai 1977). Dilly and Munch (1998) opined that the biomass-specific respiration and metabolic quotient which combines microbial activity and population is a more sensitive indication of soil pollution than is either activity or population measurements alone. Ronnpagel et al. (1998) and Brohon et al. (2001) even suggested the use of exogenous microorganisms or enzymes as bioassays for studying the bioavailability of pollutants in soils. Any reliance on microbial activity measurement needs careful consideration of the season and prevailing weather conditions, because climatic factors often determine in situ variation of soil microbial activities (Insam 1990).

6.6.1 Microbial Respiration

Microbial respiration, measured as the rate of CO_2 release or O_2 consumption, is a good general indicator of microbial activity. The basal respiration and the potential microbial respirometric activity (substrate-induced respiration) are commonly used methods for quantifying microbial respiration. Microbial respiratory activity reflects the use of energy and accordingly is a measure of the efficiency of soil organic carbon degradation (Wardle and Ghani 1995). Microflora was depressed in the presence of hydrocarbons and other agents at a contaminated site polluted with hydrocarbons for more than 50 years from leaking tanks. Measurements were made via substrate-induced respiration or potential respiratory activity, through a glucose addition method. The pollutants found included hydrocarbons (1.5–78.8 mg kg^{-1}), nickel (14.5–841.6 mg kg^{-1}) and lead (30.9–355.4 mg kg^{-1}), or cadmium (0–1.2 mg kg^{-1} of dry soil) (Brohon et al. 2001). Frey et al. (2006) found that chronic heavy metal contamination affected basal respiration in a model forest ecosystem. Generally, a negative relationship existed between the total heavy metal content and basal respiration rates of soils (Kizilkaya et al. 2004). But Van Beelen and Doelman

(1997) considered the basal respiration to be weakly sensitive to the presence of metals and organic chemical substances. Respiration rates increase with the addition of sewage sludge (Ortiz and Alcaniz 1994).

At polluted cattle dip sites in northern New South Wales, Australia, Edvantoro et al. (2003) observed that long-term contamination of arsenic and DDT led to a significant reduction in respiration, microbial biomass carbon, and fungal counts, but not in bacterial population; there was no effect in unpolluted soils. Montserrat et al. (2006) observed a delay in respiration inhibition in sludge-amended soils, depending on the waste rate used and incubation time period. The duration of exposure was long enough to allow for mineralization of oxidizable waste organic matter. In soils amended with bimetallic sludges (Mn–Zn- or Cd–Ni-rich sludge), respiration was slightly reduced in soils amended with Mn–Zn at the higher incorporation rate (6,114 and 4,900 mg kg^{-1}, respectively), whereas it was unaffected in soils with Cd–Ni-rich sludge (Renella et al. 2005). Kaufmann et al. (2006) developed a miniaturized system to test for soil respiration induced by volatile pollutants. They measured CO_2 production with a pH indicator and bicarbonate-containing agar, or the $^{14}CO_2$ evolved from ^{14}C-labeled substrates. The entire microbial respiration potential of soil is best characterized by measuring the kinetics of basal respiration, glucose-induced respiration, or cumulative oxygen consumption. This potential can also reflect the degree of biodegradation of contaminants such as organic pollutants, BTEX, or PAHs (Hollender et al. 2003). Under flooded (anoxic) conditions, microbial anaerobic respiration, which facilitates terminal electron transfer, plays an important role in transforming and degrading toxic substances. Hong and Gu (2009) reviewed bacterial anaerobic respiration and electron transfer related to the biotransformation of several pollutants in the context of soil constituents including humics, sulfonates, halogenated chemical substances, azo compounds, TNTs, metallic and nonmetallic elements.

6.6.2 Microbial Enzyme Activities

Pollutants can stimulate or suppress cellular activities in microbial communities. The important targets of pollutants are the cellular or extracellular enzymes that are present in soils. Nearly all enzymatic activities in soil are of microbial and fungal origin, and patterns of soil enzyme activity can be monitored as a measure of pollution impact (Pankhurst et al. 1995). It is important to gather knowledge on the effects that pollutants have on enzymes of living organisms, those involved in degradation of many chemically unstable pollutants, and on the commonly occurring enzyme systems in soil and water. Faber (1979) suggested that the evolution of microbial catabolic enzymes could not keep pace with the rapid introduction of environmentally persistent or recalcitrant novel compounds (e.g., halogenated hydrocarbons and complex polymers). Although the scientific literature is replete with reports on the influence of individual pollutants on different types of microbial or soil enzymes, information on the impact of mixtures of pollutants is scarce.

Kuperman and Carreiro (1997) recorded significant reductions in the activities of *N*-acetylglucosaminidase, β-glucosidase, endocellulase, and acid and alkaline phosphatases in grassland ecosystems contaminated by several metals (As, Cd, Cr, Cu, Ni, Pb, and Zn); metal levels ranged from 7.2 to 48.1 mmol kg^{-1}. Enzyme concentrations closely paralleled the levels of heavy metals. Kunito et al. (2001) studied the influences of Zn and Cu on soil enzyme activities (acid phosphatase, alkaline phosphatase, arylsulfatase, cellulase, dehydrogenase, protease, urease, β-D-glucosidase and β-D-fructofuranosidase) and microbial biomass carbon in agricultural soils amended with municipal sludge or compost since 1978. These microbial enzyme activities were sensitive to Zn stress. Among studies in which multiple trace elements were individually tested, those that generally caused the greatest inhibition in enzyme assays were Hg, Ag, Cr, and Cd (Frankenberger and Tabatabai 1991a, b; Deng and Tabatabai 1995).

Heavy metals are known to influence the activity of soil microbial communities by altering conformation of enzymes, blocking essential functional groups, or by exchanging essential metal ions (Tyler 1981; Tyler et al. 1989). Limited data are available from long-term studies on the responses of different enzymes to heavy metal contamination, especially those caused by chronic industrial pollution or acute spills under field conditions (Speir and Ross 2002). The individual and combined toxicity of trace metals (Ag, Cu, Hg, and Zn) to dehydrogenase and urease activities was regarded to potentially differ in agricultural and sandy forest soils (Chaperon and Sauve 2007). The dose–response relationships, for total soil metal content and soil solution metal content for each metal separately, were fitted to a log-logistic curve. Individually, Ag and Hg were the most efficient in reducing dehydrogenase and urease activities. Interestingly, the combined toxicity of trace metals was synergistic for agricultural soil, whereas it was antagonistic for forest soil. Higher organic matter content and higher forest soil pH were responsible for the distinctive responses. Chen et al. (2001) studied the effects of three fungicides, benomyl, captan, and chlorothalonil, on substrate-induced respiration, dehydrogenase activity, and nitrogen dynamics (NH_4–N and NO_3–N) in two laboratory experiments. Captan appeared to have a more pronounced overall effect on soil microbial activity and nitrogen dynamics than did either benomyl or chlorothalonil. Such differences on the effects of multiple pollutants on carbon or nitrogen dynamics are probably mediated by differences in enzyme responses. The simultaneous measurement of the activities of many enzymes in soils, as was suggested by Nannipieri et al. (1990), will be more useful for estimating microbial response to environmental pollution than just monitoring the activity of a single enzyme.

The potential for using soil enzyme activities as a measure for detecting soil contamination can be enhanced by combining different enzymatic activities in a multivariate analysis. Such an analysis can provide a more holistic representation of the biochemical and microbial functionality of soil (Hinojosa et al. 2004). Renella et al. (2005) performed long-term field experiments in which soil, contaminated with Mn–Zn- or Cd–Ni-rich sludge, was incorporated at two different rates (10 t dry matter (DM) ha^{-1} $year^{-1}$ or 100 t DM ha^{-1} every 2 years). These authors found that microbial biomass carbon content and soil respiration were slightly reduced in soils amended with Mn–Zn at the higher incorporation rate. But these microbial parameters were unaffected in soils receiving Cd–Ni-rich sludge. Phosphomonoesterase, β-glucosidase, and arylsulfatase activities and hydrolase-to-B_c ratios were significantly retarded in soils amended with Ni–Cd sludge at both rates, whereas the Mn–Zn sludge retarded only the arylsulfatase activity at the higher rate. Protease activity was generally more pronounced in all the sludge-amended soils than in control soils, whereas urease activity was little affected by sludge amendments. To successfully trace the outcomes, these differential responses require the application of a battery of soil enzyme tests for monitoring the effects of pollutant mixtures. Ibarrolaza et al. (2009) studied the combined effect of phenanthrene (2,000 mg kg^{-1} soil) and Cr^6 (25, 50, and 100 mg kg^{-1} soil) on bioremediation of soil contaminated with phenanthrene, dehydrogenase activity, and changes in the microbial community structure. The presence of Cr^6 at all the concentrations tested retarded the phenanthrene degradation, PAH-degrading cultivable bacteria, and dehydrogenase activity; however, these inhibitory effects were not correlated with Cr^6 concentrations. Interestingly, the water-extractable Cr^6 fraction decreased in soils co-contaminated with phenanthrene and Cr^6 compared to Cr^6 alone. Also, different Cr^6 concentrations showed different community responses to phenanthrene as evidenced by DGGE profiles.

6.6.3 Nitrification

The selection of microbial functions and species diversity for pollutant-monitoring programs can include the narrow diversity-based nitrification process (Verstraete and Mertens 2004). Most researchers believe that the long incubation times for microbial tests are less sensitive than short incubation time-based tests. The autotrophic nitrification inhibition test can be sensitive when short incubation times are used (van Beelen and Doelman 1997). In another study (Dalzell et al. 2002), IC_{50} (toxicant concentration eliciting a 50% inhibitory effect) values were determined for a number of pollutants, including single toxicants Cd, Cr, Cu, Zn, 3,5-dichlorophenol, toluene, and LAS; a standard mixture of metals and LAS; a standard mixture of organics and LAS; and 16 industrial effluents from Europe. The most sensitive bioassays included nitrification inhibition, depending on the pollutant and mixtures tested. In contrast, the nitrification test as well as the substrate-induced respiration was not affected by the levels of soil Cd (1.5–20 mg kg^{-1}), Cu (50–500 mg kg^{-1}), Ni (50–500 mg kg^{-1}), Pb (50–1,000 mg kg^{-1}), and Zn (100–1,500 mg kg^{-1}) at the contaminated sites (Murray et al. 2000).

In areas that have been impacted by pollution, the replacement of sensitive microorganisms with other more resistant species may have serious ecological consequences, because resistant microorganisms may not perform all needed ecological functions. Mertens et al. (2007) identified the Zn-tolerant nitrifying communities present in soil samples of a long-term Zn-contaminated transect toward a galvanized pylon. The authors then examined the vulnerability to addition and freeze–thaw or dry–wet cycles on the soil nitrification of the Zn-tolerant nitrifying communities to stressors such as the herbicide metamitron (4-amino-3-methyl-6-phenyl-1,2,4-triazin-5(4H)-one). The final concentration used was 100 mg L^{-1} of soil solution. Neither resistance (immediate effect) nor resilience (residual effect after 3 weeks of incubation) to these stressors was affected by adaptation of the nitrifying communities to the elevated Zn concentrations in those long-term Zn-contaminated soils. Similar observations were made by Rusk et al. (2004) and Tobor-Kaplon et al. (2005). The response of microbial community to additional stressors may thus depend on the history of soil samples and the type of stressor(s) applied (Tobor-Kaplon et al. 2006).

6.6.4 Denitrification

Nitrification and denitrification are traditionally considered to be carried out by different bacteria in segregated areas of soils. Both nitrifiers and denitrifiers are not as metabolically fastidious as previously thought; some bacteria are able to convert NH^{4+} and other reduced nitrogen compounds to nitrogen gas and the gaseous nitrogen oxides through utilization of combined nitrification/denitrification processes (Kuenen and Robertson 1994). Denitrifying bacteria represent 10–15% of the entire bacterial population in soils and can occur in threatened or polluted ecosystems (Casella and Payne 1996). Denitrifiers use a broad range of inorganic and organic compounds as sources of carbon and energy, and have an efficiency of phosphorylation equal to 67–71% of that which exists during electron transport to oxygen. Therefore, the response of denitrifiers to toxic inorganic and organic pollutants from the soil environment renders them useful for monitoring of inorganic and organic pollutants and for bioremediation.

The effect of heavy metal contamination of denitrification has been evaluated in only few studies (Bardgett et al. 1994; Gumaelius et al. 1996; Sakadevan et al. 1999). A dose-dependent heavy metal inhibition of denitrification was observed by Balderston et al. (1976), using the acetylene inhibition method. Holtan-Hartwig et al. (2002) investigated the effects of heavy metals (Cd, Cu, and Zn as two mixtures of $CdCl_2$, $CuCl_2$, and $ZnCl_2$ at 0.32, 80.00, and 120.00 and 0.64, 160.00, and 240.00 mg kg^{-1} dry soil, respectively) on the enzyme activity of a soil-denitrifying community. They also examined tolerance to the same heavy metals by quantifying the rates of nitrate reduction to N_2O and the N_2O-reductase activity. The ratio between rates of these two processes could be an indicator of the community's intrinsic capacity to release N_2O to the atmosphere. The immediate effect (after 1 day) of heavy metal exposure was a general reduction of the denitrification rate. The rate of N_2O reduction was less than the N_2O production rate. Although the N_2O production partly recovered 8 days after the introduction of the heavy metal mixture, complete restoration (equal to the control soil) was observed only after 2 months. Nevertheless, the nitrate reductase activity was not completely restored, even after 2 months. Interestingly, the exposure of extracted cells to the different heavy metals (a mixture of $CdCl_2$, $CuCl_2$, and $ZnCl_2$ at 80, 80, and 300 mg L^{-1}, respectively) led to increased Cd, Cu, and Zn tolerance of N_2O reductase activity. Henry et al. (2004) used real-time PCR to quantify the denitrifying nitrite reductase gene (*nirK*), a key enzyme of the denitrifying pathway catalyzing the reduction of soluble nitrogen oxide to gaseous form. The real-time PCR assay results for *nirK* gene were linear over seven orders of magnitude and sensitive down to 10^2

copies. The application of such sensitive molecular methods can help to determine the abundance and diversity of microorganisms. Particularly, the involvement of noncultured members within a particular physiological group of microorganisms, especially in the complex matrices of soils contaminated by the pollutant mixture, can be understood.

7 Microbial Degradation of Pollutant Mixtures

Microorganisms are capable of utilizing mixtures of pollutants for energy, as well as carbon from nutrients of natural origin, even in contaminated environments. In the 1970s, a series of experiments provided unequivocal evidence to suggest that microorganisms were indeed involved in the exceptionally rapid hydrolysis of the phosphorothioate insecticide diazinon in diazinon-retreated flooded rice paddies (Sethunathan and Pathak 1972). Later, such accelerated degradation of soil-applied insecticides by acclimatized soil microorganisms in corn fields was shown for several carbamate pesticides, the insecticide carbofuran in particular (Felsot 1989). Furukawa and Matsumura (1976) showed that the lower chlorinated members of PCB mixtures were readily degraded by *Alcaligenes* sp., and the relative proportions of higher chlorinated components such as penta-, hexa-, and hepta-chlorobiphenyls increased at the same time. When any component of the mixture is inhibitory or stimulatory to microbial activities, the pattern of degradation could change drastically. For example, mixtures containing fungicides may be persistent, largely because the fungicides suppress the metabolic activity of soil microorganisms present, although they are normally active in degrading other pesticides (Singh et al. 2002).

The ability to have high growth rates, metabolic flexibility, and adaptivity to suitable niches provide competitive advantages to pollutant-degrading microorganisms under conditions of varied nutrient availability, or even under low nutrient conditions. Most contaminated sites suffer from a sub-optimal nutrient balance and are likely to be short of nitrogen (Alexander 1999). The limitation of nutrients can prevent degradation of organic pollutants. The components of a chemical mixture can have positive or negative effects on biodegradation rates of other pollutants. The ability of microbes to utilize one substrate can enhance the degradation of a second substrate. For pure cultures, increased degradation rates of a pollutant have been reported for 2,4-dinitrophenol upon addition of glucose and for benzene and *p*-xylene upon addition of toluene (Alexander 1999). In addition, many microorganisms are capable of utilizing certain substrates only in combination with other substrates. For instance, serine cannot be utilized on its own, but only in combination with glycine, leucine, and isoleucine, when mixtures of amino acids are exposed to *E. coli* (McFall and Newman 1996). The substrate mixtures that exhibit sequential utilization during batch growth are utilized simultaneously in the case of chemostat cultures (Egli et al. 1986). Hence the substrate utilization pattern of microbial isolates in the laboratory may not reflect the catabolic capabilities of isolates under field conditions.

The microbial degradation of many natural substrate complexes is generally slow, with half-lives between 0.2 and 5 years. Xenobiotic organic contaminants such as naphthalene, phenanthrene, anthracene, and benzopyrene require a much longer time for degradation, with half-lives of 2–17 years (Litz 1992). Burback and Perry (1993) reported that *Mycobacterium vaccae* catabolized acetone, cyclohexane, styrene, benzene, ethylbenzene, propylbenzene, dioxane, and 1,2-dichloroethylene, when added singly. When present concomitantly, toluene was catabolized and benzene oxidation was delayed. The degradation of styrene was promoted by toluene but the presence of styrene led to a lower rate of toluene degradation. The degradation products of chlorobenzene and ethylbenzene (i.e., 4-chlorophenol and 4-ethylphenol) had an antagonistic effect on the degradation of other aromatic compounds by *M. vaccae*. The catabolism of pollutants to more water-soluble compounds can render them amenable to biodegradation. Nevertheless, the products of degradation may adversely affect the mineralization of other pollutants. Lin et al. (2006) conducted a batch kinetic study and determined the effect of metals on the biodegradation of MBTE in sterile mineral medium by *Pseudomonas aeruginosa*. They found that Cr^{3+} and Zn^{2+} inhibited the biodegradation of MTBE at a concentration of 1.0 mg L^{-1}. The inhibitory effect of metals at 10 and 50 mg L^{-1} concentrations on MTBE degradation followed the following order: $Cu^{2+} > Cr^{3+} > Zn^{2+} > Mn^{2+}$. The maximum specific growth rate (μ_m) of a bacterium was not affected by these metals until an MTBE threshold concentration of 10 mg L^{-1} was reached. In contrast, a significant decline in the bacterial growth rate was observed for Cu even at 1.0 mg L^{-1}. Raynal and Pruden (2008) studied the influence of BTEX on the biodegradation of MTBE in batch and semi-batch reactors inoculated with MTBE-degrading cultures that were enriched with MTBE only and MTBE plus BTEX. They found that BTEX inhibited MTBE degradation in batch reactors, compared to semi-batch reactors wherein there was no accumulation of BTEX intermediates.

The presence of heavy metals as co-contaminants with organic compounds has been shown to inhibit organic degradation processes (Sandrin and Maier 2003). Cadmium at 60 mg kg^{-1} has been shown to inhibit 2,4-dichlorophenoxyacetic acid (2,4-D) degradation in a soil inoculated with a 2,4-D-degrading bacterium, *Ralstonia eutropha* strain JMP134 (Roane et al. 2001). However, coinoculation of soil with JMP134 and the cadmium-resistant and bioaccumulating bacterium *Pseudomonas* H1 effected rapid degradation of 2,4-D (Roane et al. 2001). Pardue et al. (1996) studied the effect of cadmium on dechlorination of trichloroaniline (model co-contaminant) in three anaerobic sediments such as a freshwater marsh, cypress bottomland hardwood, and a rice paddy. Dechlorination was inhibited in all three sediments; however, the inhibition was dependent on the soluble cadmium in pore water and its speciation. Liu et al. (2008) reported that the combination of cypermethrin and copper severely retarded soil catalase activity more than did either chemical alone. They suggested that the lipophilic nature of cypermethrin could have affected membrane permeability, thereby enhancing entry of Cu ions into the cell.

Heavy metals (Cu, Hg, Zn, Cd, and Cr) exert harmful effects on the biodegradation of organic xenobiotics, i.e., the herbicide 2,4-D methyl ester (2,4-DME) (Said and Lewis 1991), 2-chloro-phenol, 3-chlorobenzoate, phenol, benzoate (Kuo and Genthner 1996), 3-chloroethylene (DeGraffenreid and Shreve 1998), naphthalene (Malakul et al. 1998), and diesel fuel (Riis et al. 2002), as observed in cultures, laboratory microcosms, sediments, or fields. The presence of copper in soil is reported to reduce the biodegradation of organic pesticides (Said and Lewis 1991; Birch and Brandl 1996). Wenderoth and Reber (1999) showed that the average number of aromatic acids utilized by bacterial isolates, among 21 substrates tested, decreased if the soils were contaminated by metals (predominantly by Zn from application of sewage sludge). Inoculation of arsenic methylating fungi into cattle dip soil contaminated with arsenic (1,390 mg As kg^{-1}) accelerated the rate of arsenic volatilization (Edvantoro et al. 2004). However, the arsenic volatilization rate by these fungi declined when the same concentration of arsenic was present in combination with DDT at 194 mg kg^{-1}. This suggests that DDT inhibits the rates of microbial formation of arsine in the contaminated dip soils. Recently, Joshi et al. (2008) identified a novel bacterial consortium consisting of *Aeromonas caviae, Proteus mirabilis*, and *Rhodococcus globerulus,* which could decolorize Acid Orange 7 (AO7) and 15 other azo dyes individually, as well as a simulated wastewater containing a mixture of all the 16 azo dyes. The mutual interaction that exists among bacterial strains or species confers the possibility that they could be applied as a consortium to treat industrial wastewaters or other wastes. Swindell and Reid (2006) investigated the effect of diesel fuel on the fate of $[^{14}C]$phenanthrene (21 mg kg^{-1} soil) in soil. They found that diesel fuel, up to a level of 2,000 mg kg^{-1} soil, resulted in an increase in phenanthrene loss. However, 20,000 mg kg^{-1} diesel fuel retarded degradation of phenanthrene. The suggested reason for this was the reduced partitioning of phenanthrene from the nonaqueous phase liquid (NAPL) to aqueous phase, resulting in reduced biodegradation.

Different requirements and conditions may exist for the degradation of an individual component within a mixture of pollutants. Spatial and temporal changes in the contaminants may be influenced by the physico-chemical properties or stability of the chemicals present, which, in turn, may affect the environmental fate of pollutant mixtures. Organometallic compounds show unique toxic effects, even at very low concentrations, and these toxic effects depend on the nature and number of organic groups bound to metallic cations. In PAH (flourene, anthracene, pyrene, and chrysene)-contaminated soil, heavy metals (Zn, Pb, and Cd) penetrated more easily into cells and produced more pronounced effects on microbial cell functions (Maliszewska-Kordybach and Smreczak 2003). In addition, inorganic compounds can aid in the degradation of many organic pollutants. Manganese and iron oxides and some clay minerals (e.g., smectites containing Fe(III)) promote the oxidation of many organic pollutants (Mortland and Halloran 1976; Pizzigallo et al. 1995). Nasser et al. (2000) also showed that the degradation of herbicides such as 2,4-D and atrazine could be achieved by grinding them gently (by hand with agate mortar) with manganese oxide.

8 Microbial Biosensors and Indicators

Several useful biosensors and indicator tests exist that are useful in studying microbes and how they respond to pollution. Some of these tests are useful in studying the effect of pollution mixtures on microbes or microbial processes. Some of

Table 7 List of selected microbial indicators and methods used for monitoring pollutant mixtures

Microbial indicator	Method	Scale of enquiry
Microbial biomass	Microscopy/indirect methods	Microbial biomass
Microbial quotient	C_{micro}/C_{org}	Microbial biomass
Fungal–bacterial ratio	PLFA	Microbial biomass
Genetic diversity	PCR-based methods	Community diversity
	(DGGE/T-RFLP)	Community diversity
Functional diversity	BIOLOG^{TM}	Community diversity
Marker lipids	PLFA	Community diversity
Bacterial DNA synthesis	Thymidine incorporation	Microbial activity
Bacterial protein synthesis	Leucine incorporation	Microbial activity
Soil respiration	CO_2 production or	C-cycling
	CO_2 consumption	C-cycling
Decomposition of organic matter	Litter bags	C-cycling
Soil enzyme activity	Enzyme assays	C-cycling
Methane oxidation	Methane measurements	C-cycling
N-mineralization	NH_4^+ accumulation	N-cycling
Nitrification	NH_4^+ oxidation assay	N-cycling
Denitrification	Acetylene inhibition assay	N-cycling
N_2 fixation	Nitrogenase assay	N-cycling
Biosensor bacteria	Microtox®	Bioavailability
Catabolic genes	Selective culture medium	Bioavailability

the main biosensors and indicator tests can be used for monitoring environmental pollution, and these are presented in Table 7.

The standard biological assay for environmental pollution is the microbial multiplication inhibition test. In this test, the degree of growth inhibition of a microbial population is measured after exposure to a chemical. Single-species microbial tests and bioassays provide a better understanding of the mechanistic effects of pollutants. The luminescent bacteria test (*Vibrio fischeri*, Microtox) or the genetically modified (*lux*-marked) bioluminescent bacteria test is used in ecotoxicity assessment (Paton et al. 1997; Bundy et al. 2001). Bioluminescent microbial biosensors are used to detect chromate–copper–arsenate (Cai and DuBow 1997), Ni^{2+} and chromate (Peitzsch et al. 1998), and antimonite and arsenite (Ramanathan et al. 1997). Wang et al. (2009) found that the toxicity of the mixture of copper and a PAH, phenanthrenequinone, measured in terms of bioluminescence, to the bacterium *V. fischeri* depended on the ratio of the pollutants in the mixture. Thus, an increase in copper concentration relative to PAH increased the toxicity of the mixture. The interaction was antagonistic at low Cu:PAH ratio (1:4), whereas the nature of the interaction changed to additive at an intermediate ratio (2:3 of Cu:PAH), and finally to a synergistic interaction at a high Cu:PAH ratio (3:2 and 4:1). One explanation is that the shift in the type of interaction observed derives from Cu being an essential metal at low concentrations, but becomes toxic at high concentrations. Interestingly, the toxicity of Cd in combination with PAH did not depend on the mixture ratio. The ratio of luminescent bacteria to total heterotrophic bacteria (plate counts) is considered to be a useful indicator of anthropogenic impact in estuarine ecosystems (Ramaiah and Chandramohan 1993; Frischer et al. 2005). The suppression of the luminescence phenotype by a large number of diverse pollutants has been exploited in commercial assays to monitor for various organic and inorganic chemical contaminants in a wide variety of environments (Doherty 2001). Girotti et al. (2008) reviewed the characteristics and applications of natural bioluminescent bacteria, especially for detecting organic and inorganic pollutants.

The ubiquitous presence of pollutants necessitates the monitoring of soil health, either through chemical or biological indicators. Leitgib et al. (2007) evaluated the applicability and reliability of several environmental toxicity tests, by comparing the result with whole soils and their water extracts. The authors measured the following endpoints: (a) the bioluminescence inhibition in *V. fischeri* (bacterium), (b) the dehydrogenase activity inhibition in *Azomonas agilis* (bacterium), (c) the reproduction inhibition in *Tetrahymena pyriformis* (protozoon) and *Panagrellus redivivus* (nematode), (d) the mortality in *Folsomia candida* (springtail), (e) the root and shoot elongation inhibition in *Sinapis alba* (white mustard, a plant), and (f) the nitrification activity inhibition in an uncontaminated garden soil for three different contaminated sites of organic and inorganic pollutants. One of them was polluted with transformer oil (PCB-free), the second with mazout, and a third with toxic metals such as Zn, Cd, Cu, Pb, and As. The authors suggested that the interactive ecotoxicity tests indicated a more harmful effect with the contaminated soil than with the corresponding soil extracts. There are some recent applications of microbial biosensors which make use of electrochemical, optical, acoustic, or electronic transducers and the genetically engineered microorganisms, based on fusing of the *lux*, *gfp*, or *lacZ* gene reporters to an inducible gene promoter in environmental monitoring of pollutants (D’Souza 2001; Lei et al. 2006).

Nielsen et al. (2002) qualified a microbial parameter as microbial indicator when that parameter could interpret and represent the properties of or impact on the environment. To easily quantify the influence of pollutants on microbe-mediated processes in soil, Babich et al. (1983) developed the concept of an “ecological dose 50%” (ED_{50}). The ED_{50} is the concentration of a toxicant that inhibits a microbe-mediated ecological process by 50%. Doelman and Haanstra (1986) applied the concept of ED_{50} to determine the factors that influence the speciation and mobility of heavy metals in soils and control their availability and residence time. Speir et al. (1995) used the ED_{50} to establish which microbiological and biochemical properties of soil were most sensitive to heavy metal contamination. Different mathematical models are used to calculate ED_{50} values. The Michaelis–Menten kinetic approach was used to model inhibition of soil biological properties by Cr(VI) (Speir et al. 1995) and As(V) (Speir et al. 1999), respectively. The sigmoidal dose–response model relating the tested parameters to the natural logarithm of the heavy metal concentration was used by Haanstra et al. (1985). Kools et al. (2005) introduced the concept of degradation rate of glyphosate as an indicator for ecosystem health to assess the pollution in soils with a long history of heavy metal pollution (Cu, Pb, and Zn). A positive correlation existed between the rates of glyphosate degradation and the soil metal pollution.

9 Future Outlook

Both industrial and agricultural chemicals generally exist as mixtures and contribute to soil contamination. Neither determining the toxicity of a mixture of contaminants nor the degree to which there are interactive effects (additive, synergistic, or otherwise) at a polluted site is a simple task. It is also difficult to establish what contaminant(s) at a mixed-contaminant polluted site are initially responsible for causing a toxic effect. The complexity derives from the fact that the compounds present may be metabolically altered to metabolites that become nontoxic or more toxic, or the parent compounds may exert a direct toxic action on biota. Moreover, microorganisms at a polluted site may be subjected to the synergistic or antagonistic effects of exposure to the chemical cocktail present. In certain environments, toxicants can produce shifts in microbial populations that adversely affect ecosystem function and processes. The natures of the interactions that occur in a chemical mixture depend significantly on the properties of the soil, the organisms, and the receiving environment in which they are exposed. The guidelines we now use for performing chemical risk assessments that involve "additivity" do not always reflect the real risks of complex mixtures. Thus, depending on the properties of the soil, nature of the chemicals involved, organisms present, contaminant bioavailability, and dosage, the toxicity of a chemical mixture may exhibit antagonistic, synergistic, or no interaction. Hence having toxicological data on the mixtures present at mixed-contaminant sites is critical to informed decision making by regulators or others. Currently, the lack of such information is a major hurdle in conducting ecological risk assessment at such sites.

The long-term monitoring of many different ecosystems is essential for collecting information on pollution patterns and the changes that occur in the context of environmental reclamation or remediation. Although various monitoring methods and microbial indicators are presently available, more refined new methodologies are needed to meet future pollutant abatement challenges. Although it is easier to obtain scientific information on the effects of an individual pollutant or an individual microorganism, it will be only through creation of newer strategies and tools, such as molecular fingerprinting of microbial communities, that we will improve risk assessment for complex chemical mixtures. One future area of promise is to use new toxicological data on environmentally relevant contaminant mixtures to develop mathematical models and simulation studies for achieving a better understanding of the distribution and toxicity of pollutant mixtures. Moreover, the application of such models can significantly reduce experimental and chemical analysis costs.

Microorganisms are, and will continue to be, the most important agents for mediating degradation and detoxification processes that effectively remove chemical pollutants from the soil and aqueous environments. They not only are diverse in their physiological capabilities but also employ different strategies that allow them to utilize certain chemical pollutants. There are several factors which render microbial indicator strains to be much sought after. Among these are the following: microbes have large genomes, redundant, and versatile catabolic pathways, an ability to take up and metabolize hydrophobic compounds, an ability to form biofilms, and an ability to survive under adverse conditions. In addition, new tools for genetic engineering of microbes have recently become available. Recent advances made in bioremediation strategies will make the use of molecular, genetic, and protein engineering tools increasingly attractive. The next generation of improvements in bioremediation will include identification of novel metal-sequestering peptides, rational and irrational pathway engineering, and enzyme design. These advances are expected to improve cost effectiveness and may improve the specificity for removal of pollutants. Though environmental pollution is best abated by acting against the offending pollutants, the superior policy is *prevention is better than cure*.

Future research should also be directed toward (i) generating scientific data on the toxicity of commonly occurring contaminants at their environmentally relevant concentrations, (ii) improving risk assessment methods to reduce uncertainty, (iii) achieving more balance when studying whole mixtures vs. interaction of individual chemicals in a mixture, and (iv) performing more exposure assessments with improved methods that involve multi-species testing.

10 Summary

Soil is the ultimate sink for most contaminants and rarely has only a single contaminant. More than is generally acknowledged, environmental pollutants exist as mixtures (organic–organic, inorganic–inorganic, and organic–inorganic). It is much more difficult to study chemical mixtures than individual chemicals, especially in the complex soil environment. Similarly, understanding the toxicity of a chemical mixture on different microbial species is much more complex, time consuming, and expensive, because multiple testing designs are needed for an increased array of variables. Therefore, until now, scientific enquiries worldwide have extensively addressed the effects of only individual pollutants toward nontarget microorganisms. In this review, we emphasize the present status of research on (i) the environmental occurrence of pollutant mixtures; (ii) the interactions between pollutant mixtures and ecologically beneficial microorganisms; and (iii) the impact of such interactions on environmental quality. We also address the limitations of traditional cultivation-based methods for monitoring the effects of pollutant mixtures on microorganisms. Long-term monitoring of the effects of pollutant mixtures on microorganisms, particularly in soil and aquatic ecosystems, has received little attention. Microbial communities that can degrade or can develop tolerance to, or are inhibited by, chemical mixtures greatly contribute to resilience and resistance in soil environments. We also stress in this review the important emerging trend associated with the employment of molecular methods for establishing the effects of pollutant mixtures on microbial communities. There is currently a lack of sufficient cogent toxicological data on chemical mixtures for making informed decision making in risk assessment by regulators. Therefore, not only more toxicology information on mixtures is needed but also there is an urgent need to generate sufficient, suitable, and long-term modeling data that have higher predictability when assessing pollutant mixture effects on microorganisms. Such data would improve risk assessment at contaminated sites and would help devise more effective bioremediation strategies.

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Fluoride in the Environment and Its Metabolism in Humans

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Contents

1 Introduction

Fluorine is widely dispersed in nature and is estimated to be the 13th most abundant element on our planet (Mason and Moore 1982). It is the most electronegative of all chemical elements, and as a result, it never exists in elemental

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form, but rather combines with other elements; fluoride compounds represent about 0.06–0.09% of the content of the earth's crust (Wedephol 1974). Fluoride is distributed universally throughout soils, plants, and animals, and is assumed to be an essential element in animals, including humans. Fluoride has an important role in bone mineralization and formation of dental enamels. Fluoride, when consumed in inadequate quantities (less than 0.5 ppm), causes health problems such as dental caries, lack of formation of dental enamel, and reduced bone mineralization, especially among children (WHO 1996). In contrast, when fluoride is consumed in excess (more than 1 ppm), health problems may result, which equally affect the young and old (WHO 1996). At higher fluoride concentrations, metabolic processes are affected in humans, and overexposed individuals may suffer from skeletal or dental fluorosis, non-skeletal manifestations, or combinations of these maladies (Susheela et al. 1993). The incidence and severity of fluorosis depends upon the fluoride concentration in air, soil or water, and the degree of exposure to these levels.

Among the three forms of environmental media (air, soil, and water), groundwater is the major source of fluoride exposure in humans. To sustain life, freshwater must be continuously available to humans. Throughout history, humans have relied on groundwater as a source of drinking water, and even today, more than half of the world's population depends on sources of groundwater for survival. Groundwater constitutes 97% of total global freshwater, and in many regions, groundwater sources constitute the single largest available supply of fresh drinking water (WHO 2004). When the rates of groundwater extraction exceed recharge rates, depletion of this valuable resource occurs, with a resultant restriction of the drinking water supply.

Fluoride is one of the most significant of groundwater contaminants and is of concern primarily because it has both short- and long-term effects on human health. Moreover, in many regions of the world, it is very difficult to avoid exposure to fluoride. Although groundwater quality may be impaired by many natural constituents, fluoride is among the most prominent pollutants, because its geogenic origin renders it so widespread.

Fluorosis has been documented to have produced significant health impairment to the citizens of more than 25 nations across the world (UNICEF 1999). The number of people who suffer from fluoride poisoning is increasing daily. It has been estimated that more than 200 million people worldwide are at risk of fluorosis (UNICEF 1999). In India, about 80% of domestic freshwater needs in rural areas and 50% in urban areas are met by groundwater. The individuals who rely on tube wells for freshwater are under threat from continuing exposure to excess fluoride, arsenic, iron, nitrate, and salinity.

The levels of natural fluoride that occur in groundwater range from 0.5 to 48 ppm, or more (Susheela 2003). The presence of even low levels of fluoride in groundwater, when coupled with a general prevalence of malnutrition, may turn disastrous, especially for children in rural and semi-urban areas of India or elsewhere. Over the past few decades, the prevalence and severity of fluorosis has increased radically in India, reaching near epidemic levels. At present, 20 of 35 states and

Union Territories are facing excessive fluoride intakes, whereas new areas are also being affected by this problem. Because of excess fluoride in groundwater, WHO reduced the permissible limit in drinking water of fluoride in India from 1.5 to 1.0 ppm in the year 1998 (UNICEF 1999). Fluoride can be detected in drinking water only by laboratory analysis, because water contaminated with fluoride is colorless, tasteless, and odorless. As a result, victims discover that they have been consuming fluoride-contaminated water only after manifestations of fluoride disease surfaces.

Common symptoms of fluoride toxicity in humans are stained teeth, paralyzing bone disease, stooped backs, crooked hands and legs, blindness, and other deformities. In fluoride-endemic areas, fluoride exposure not only cripples the limbs of children and adults but also cripples their hopes. Excess fluoride contamination of drinking waters has now acquired the dimensions of a socio-economic problem, in addition to being a public health problem. The United Nations had proclaimed the decade from 2005 to 2015 as the “Decade of Water for Life”; still, millions of peoples lack safe drinking water. Solutions are needed to mitigate the sufferings of people who lack access to safe drinking water, as well as those who may be affected by fluoride-induced disease. But mitigation of the problem is possible only when the areas that are potentially affected by fluoride contamination have been adequately delineated and mapped.

The main objective of this chapter is to provide readers with an overview of the key factors that are responsible for fluoride exposure, including the problems caused by such exposure, and a description of how fluoride is absorbed and metabolized in humans. We have also emphasized, in this review, aspects of the chemistry of fluorine, the source and occurrence of fluoride in the environment, and how it is distributed in soil, water, and plants.

2 The Chemistry of Fluorine

Fluorine is an element of the halogen group and is placed at VIIA in the periodic table. Fluorine exists as a light yellow green, pungent gas, whose atomic number is 9 and molecular weight is 18.998 g/mol. It is univalent, and in many compounds, the stereochemistry of the fluoride ion resembles that of OH, because their ionic radii are similar (F 133 pm, OH 140 pm) (O’Donnell 1975). It is the most electronegative element in the periodic table (4.0 in the Pauling scale) (Neumuller 1981). Inorganic fluoride is either free or matrix bound in minerals, or covalently bound in various inorganic or organic compounds. Because the electronic configuration of fluorine is $1s^2,2s^2,2p^5$, fluorine needs to gain only one electron in its outer shell to form a stable inorganic fluoride ion. Doing so allows the atom to attain an inert gas configuration. The high reactivity (i.e., oxidizing capacity) of fluorine basically results from its high electro-negativity, its unusually low dissociation energy, and the great bond strength of the compounds it forms. Because of its high reactivity, fluorine never exists free in nature but is always present in a combined form with other elements. It reacts vigorously with every other element except the inert gases. The environmental abundance of inorganic fluoride is increasing, because it is increasingly released into the environment as waste from use in industrial and dental health products.

3 Environmental Sources and Mobility of Fluoride

3.1 Natural Sources

In nature, fluorine is widely found in sedimentary phosphate rock deposits and minerals (Kirk-Othmer 1980). The main fluorine-containing minerals are fluorspar or fluorite, fluorapatite, and cryolite. For industrial purposes, the most important source of fluorine is mineral calcium fluoride (fluorite or fluorspar, CaF_2), which has a fluorine content of 49% (Fuge 1988). Fluorite, commercially known as fluorspar, is usually associated with quartz, calcite, dolomite, or barite (Prud'homme 1990). Although the largest amount of fluoride exists as fluorapatite [$Ca_5(OH,F)(PO_4)_3$], this mineral is mined mainly for its low inorganic fluoride (4% fluorine) (Kirk-Othmer 1980). By comparison, cryolite (Na_3AlF_6) is rare (54% of F). In addition to CaF_2, fluorapatite, and cryolite, a number of other silicates, such as topaz ($Al_2SiO_4(OH,F)_2$), oxides, carbonates, sulfates, phosphates, sellaite (MgF_2), and sodium fluoride or villiaumite, contain minor amounts of inorganic fluoride (Fuge 1988). Host minerals, such as mica (layer silicates), amphiboles (chain silicates), apatite, and tourmaline, and clays, such as montmorillonite, kaolinite, and bentonite, also contain inorganic fluoride (Reimann and Decaritat 1998). Fluorine may occur in limestone that is associated with tremolite, actinolite, and pyroxene, where fluoride concentrations may reach a level of 0.4–1.2%. A part of the fluorine may be present in clay material admixed with weathered limestone. The content of fluoride in various rock types is presented in Table 1.

Table 1 Fluoride content in various types of rocks

Rocks	Fluoride range (ppm)	Average (ppm)
Basalt	20–1060	360
Granites and gneisses	20–2700	870
Shales and clays	10–7600	800
Limestones	0–1200	220
Sandstones	10–880	180
Phosphorite	24000–41500	31000
Coal (ash)	40–480	80

Source: Keller (1979)

3.2 Anthropogenic Sources

In most developed and developing countries, the native soil fluoride content is strongly affected by the application of fertilizers and by deposition of industrial airborne pollutants. Several anthropogenic sources enrich the fluoride content of soil. Important emission sources of fluoride include coal burning, oil refining, steel production, chemical production, clay production, Al smelting, glass and enamel manufacture, brick and ceramic manufacturing, distribution of fluoride-containing fertilizers and pesticides, wastes from sewage and sludges, production of uranium hexafluoride (UF_6), and uranium trifluoride (UF_3) from the nuclear industry (Neumuller 1981; Fuge 1988; Fuge and Andrews 1988).

Phosphatic fertilizers, especially the super-phosphates, are perhaps the single most important sources of fluoride contamination to agricultural lands. A repeated application of rock phosphate that contains several percent of fluoride was shown to significantly elevate the fluoride content of soils (Omueti and Jones 1977). Typical additions of phosphatic fertilizer (50–100 kg P_2O_5/ha/yr) may elevate soil fluoride content by 5–10 ppm/yr (Gilpin and Johnson 1980). Rock phosphates generally contain approximately 3.5% of fluoride; phosphatic fertilizers contain between 1.5 and 3.0% fluoride (McLaughlin et al. 1996). Labile and water-soluble fluoride concentrations appear to be most affected by industrial pollution (Polomski et al. 1982; Haidouti 1991). The surface soil in the vicinity of brick field industries found to contain water-soluble fluoride (1:1) ranged from 0.59 to 2.74 ppm; $CaCl_2$ extractable fluoride ranged from 0.69 to 3.18 ppm, whereas the mean total fluoride concentration varied from 322 to 456 μg/g (Jha et al. 2008). The prolonged application of super-phosphate can also have marked effects on total soil fluoride content (Kudzin and Pashova 1970).

3.3 *Distribution and Mobility of Fluoride in Soils*

The fluoride native to soil is generally contained within several minerals, most commonly apatite [especially fluorapatite ($Ca_5(PO_4)_3F$, fluorite (CaF_2), cryolite (Na_3AlF_6)], forms of topaz ($Al_2(SiO_4)F_2$), and within micaceous clay minerals. Fluoride is also present in soil as specifically and non-specifically adsorbed ions and compounds (Bowen 1966; Pickering 1985). The native soil fluoride level is highly dependent on the parent material from which the soil is formed, whereas its distribution in the soil profile is a function of soil-forming processes, of which the degree of weathering and clay content are the most pronounced. Very common soil minerals, such as biotite, muscovite, and hornblende, may contain as much as several percent of fluoride and therefore are the main source of fluoride in soil.

Fluoride has been lost from the surface horizons of most soils; hence, it is not surprising that organic matter has a low affinity for fluoride. Omueti and Jones (1980) gave the range of fluoride concentration in organic matter of the surface horizon to be as low as 0.03–0.12 ppm. In almost all studies of the fluoride content of soils that are uncontaminated, high variability has been reported. The average fluoride content of soil, worldwide, has been calculated to be 320 ppm (Kabata-Pendias and Pendias 1984). The total fluoride concentration in normal arable soils ranges from 150 to 360 ppm but can reach up to 620 ppm. Many authors correlate the variability of total soil fluoride content to the particle size of the soil, in particular, increasing amounts of total fluoride are associated with increasing clay content (Robinson and

Edington 1946; Omueti and Jones 1977). Under normal conditions, it is also common for total soil fluoride content to increase with soil profile depth. This may result from the low affinity that fluoride has for organic matter (Omueti and Jones 1977). Increasing soil fluoride content, with increasing depth, may also be due to long-term downward movement of fluoride through the soil profile. The labile forms of soil fluoride are variously estimated to be in the form of water-soluble, acid-extractable, and resin-extractable fluoride compounds.

Fluorine forms its most stable bonds with Fe, Al, and Ca, whereas labile fluoride is held by soil components that include clay minerals, Ca and Mg compounds, Fe and Al compounds (Bower and Hatcher 1967). At low concentrations, Fe and Al oxides and hydroxides have the greatest ability to absorb fluoride. In natural soil solution, the fluoride concentrations are normally a small proportion of labile soil fluoride and normally are <1 μg/ml. In almost all soils, fluoride is strongly bound, and only in coarse clay and Fe/Al oxide-poor soils is the fluoride weakly held (Pickering 1985).

Many common fluoride compounds are only sparingly soluble, e.g., CaF_2 (0.016 g/L), MgF_2 (0.13 g/L), and Na_3AlF_6 (0.42 g/L), although some others are quite soluble, e.g., HF, SiF_4 (hydrates), and NaF (40 g/L) (Pickering 1985). Several investigators have observed that the solubility of fluoride in soils is highly variable and has the tendency to be higher at pHs below 5 and above 6. The solubility of fluoride tends to be lowest in the pH range of 5–6.5, which coincides with the greatest fluoride sorption (Wenzel and Blum 1992). Fluorine solubility in soil is complex and may be controlled by solid phases. In addition, F solubility may be related to the solubility of Al or other ionic species with which it forms complexes. At low pH levels, complexes are formed between Al and F in soil solution, and little is present as free F^- ions. At high pH, an increasingly unfavorable electrostatic potential decreases the retention of the fluoride ion to soil and increases the F concentration in soil solution. The increase of fluoride in the soil solution may also result from displacement of adsorbed fluoride, i.e., by the increased concentration of OH^- in soil solution at higher pHs (Larsen and Widdowson 1971). Thus the mobility of fluoride in soils is complex. The predominant factors controlling the level of the F ion in the soil solution are the amount of clay minerals present, the soil pH, and the concentration of Ca and P in the soil. In general, the greatest adsorption of F by soil mineral components is either at the distinct acid range of the pH scale or at about pH 6–7 (Fig. 1).

Mobile or water-soluble fluoride is easily adsorbed by clay and phosphates. At lower pH levels, sorption decreases due to the formation of soluble Al–F species such as $(AlF)^{2+}$ and $(AlF_2)^+$ complexes (Barrow and Ellis 1986; Wenzel and Blum 1992). On the other hand, in soils having high pH and low levels of amorphous Al species, clay surfaces and OM generally sorb little F (Omueti and Jones 1977). At alkaline pHs, the increased negative surface charge results in repulsion of anionic F. Under such conditions, the predominant retention mechanism is the exchange of F with OH groups of amorphous materials, such as Al hydroxide. In this case, the crystal lattice OH of clay minerals is replaced by F, resulting in a simultaneous release of Al and Fe. Another F retention mechanism involves F precipitation

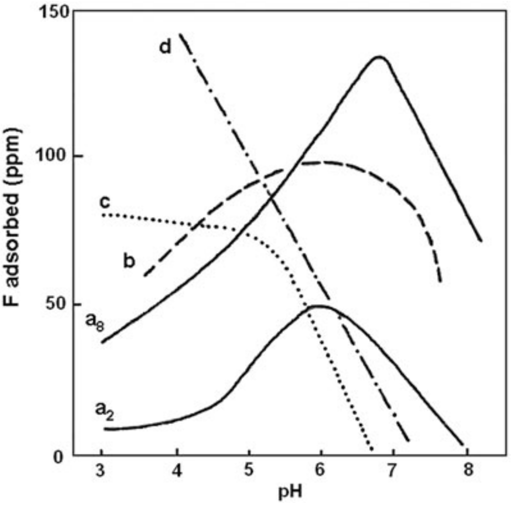


Fig. 1 Adsorption of fluoride by soil and clay as a function of pH (a_2 and a_8 soil equilibrated against 2 and 8 mg F/L solutions, respectively): b, bentonite; c, bauxite; and d, kaolinite. All clays equilibrated against a 2 mg F/L solution (Omueti and Jones 1977)

as CaF_2 in calcareous soils (Slavek et al. 1984). At neutral to alkaline pH levels, F exists predominantly as the F^- ion, and at a pH of < 5.5, the fluoride is complexed with Al (Wenzel and Blum 1992). The adsorption of F over a range of 2–16 mg/L was well described by Langmuir isotherms and the pH of the soil solution phase consequently increased, although the increase in OH^- was only a small fraction of the adsorbed F. The order in the ability of various materials to absorb F was as follows: $Al(OH)_3$ precipitate on bentonite> $Al(OH)_3$ >> hydrated hallosite and dehydrated halloysite > a weakly acidic soil >> kaolinite > gibbsite > alkaline soil > goethite > bentonite and vermiculite.

$Al(OH)_3$ has an extremely high F adsorption capacity. The F adsorption occurs primarily by exchange with OH groups from $Al(OH)_3$, and basic Al polymers adsorbed on mineral surfaces, rather than by exchange with crystal lattice OH group of clay minerals. In calcareous soils, the formation of slightly soluble CaF_2 and F complexes with Al, Fe, and Si is responsible for the low migration of this element. In sodic soils, on the other hand, high levels of exchangeable Na effects increased solubility of F. Chhabra et al. (1980) had also reported a linear increase of water-soluble fluoride with the increase of exchangeable sodium percentage (ESP).

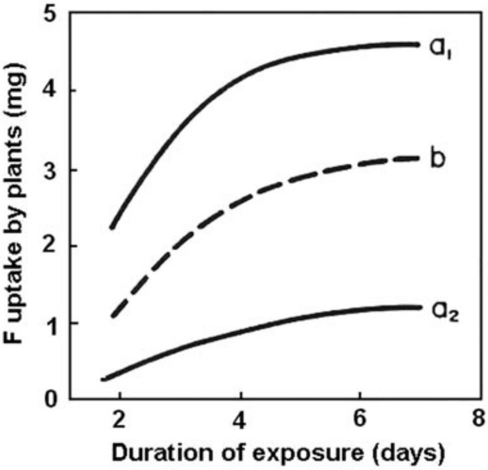
4 Distribution of Fluoride in Plants and Soil

Fluoride is not an essential plant element but is essential to animals and humans. However, continuous ingestion of excessive amounts of fluoride may lead to the disorder “fluorosis,” whereas sub-optimal levels in the diet can have an equally damaging effect in other ways. Therefore, the fluoride content in plants is of interest not only to humans but also for animals and to livestock producers (Keerthisinghe et al. 1991).

4.1 Fluoride Absorption and Mobility in Plants

The availability of fluoride to plants is usually not related to the total fluoride or soluble fluoride content of the soil in which they grow. However, under certain soil and plant conditions, the F content of plants seems to reflect its occurrence in soils (Shupe and Sharma 1976). Bieliyakova (1977) gave the ratio of fluoride in plant ash to fluoride in soils as 0.2 and 0.6 for cultivated and natural vegetation, respectively. These values indicate a relatively low F bioavailability to plants. The soluble F fraction in soil is taken up passively by roots and apparently is easily transported in plants. Although it has been shown that plants can take up F quite easily from polluted soils, the bioavailability of soluble fluoride is of much less significance than that from airborne compounds. In other words, when fluoride is present as both an air pollutant and a soil pollutant, the F uptake by plants from air is much more significant than is the uptake from soil. Several factors affect plant accumulation of airborne fluoride, but the most pronounced are atmospheric fluoride concentration and the duration of exposure (Fig. 2).

Fig. 2 F uptake by plants as a function of F contamination in air and of exposure time; a_1 and a_2 represent F concentration at 11 and 5 μg/m^3, respectively, in bean plants, whereas b represents an F concentration of 5 μg/m^3 in tomato plants



Gaseous fluoride enters the leaf through stomata and then dissolves in the water that permeates the cell walls. The natural flow of water in a leaf is toward the sites of greatest evaporation, which are the leaf margins and tips. Carried by water, fluoride concentrates in the margins and tips, so these areas are generally the first to show visual injury. Generally, leaves are the most sensitive when they are young and still growing. Once fully developed, leaves may be many times more resistant to F. Exposure to a high F concentration causes necrosis of leaf parts or even the whole of the leaf.

4.2 Critical Level of Fluoride in Soil

Increased fluoride uptake is toxic to plants and animals, thus it is desirable to minimize the rates of fluoride accumulation in soils. It is a difficult task to establish a single upper threshold concentration for F toxicity to plants, because different soils differ in their ability to retain fluoride. Hence a high concentration of fluoride is strongly retained in a fine-textured amorphous Al hydroxide-rich soil. However, a much lower concentration is retained in a very coarse-textured calcareous soil that is low in amorphous Al compounds and therefore may damage plants.

Plants are known to take up F, but the degree to which this occurs varies among plant species (Brewer 1966). In soil solution, different chemical species of fluoride exists. In acidic soils, a significant fraction of fluoride exists as AlF^{2+} and $AlF_2{}^+$ (Manoharan et al. 1996). The Al–F species are non-toxic to plants at lower concentrations; this species is variably toxic at higher concentrations, depending on species (Manoharan 1997; McLaughlin et al. 1997). Stevens et al. (1997) showed that Al–F species are less toxic than are Al^{3+}, $Al(OH)^{2+}$**,** and $Al(OH)_2{}^+$ species to tomato and oats.

Around the globe, at present, there are no regulations or even recommendations as to what the permissible limit of F in soils should be. The reason may be the paucity of the data available for F content of soils worldwide and/or the wide variation that exists in reported fluoride soil levels.

5 Occurrence of Fluoride in Groundwater

Water is an essential natural resource for sustaining life and is among nature's most valuable gifts. Once viewed as an infinite and bountiful resource, today, water often defines the limits of human, social, and economic development for a region. The main source of freshwater for sustaining life on earth is groundwater. Unfortunately, groundwater is either being increasingly depleted for irrigation of crops, industrial, or other uses, or is becoming contaminated by various pollutants. The presence of fluoride as a contaminant of groundwater has become a worldwide problem, because it is commonly found in groundwater sources. The problem of high fluoride content in groundwater resources is important, because of both toxicological and geoenvironmental concerns.

The fluoride that contaminates groundwater derives mainly from natural phenomena but is influenced by the nature of local and regional geology and the existence of certain hydro-geochemical conditions. The chief source of fluoride in groundwater is fluoride-bearing minerals that exist in rocks and soils. The weathering and aqueous leaching processes that occur in soils play an important role in determining the amounts of fluoride that reaches groundwater. The various factors that govern the release of fluoride into water from fluoride-bearing minerals are (i) the chemical composition of the water, (ii) the presence and accessibility of fluoride minerals to water, and (iii) the contact time between the source mineral and water (Keller 1979).

Overall water quality (e.g., pH, hardness, and ionic strength) also plays an important role by influencing mineral solubility, complexation, and sorption/exchange reactions (Apambire et al. 1997). The alkaline condition of groundwater favors increased solubility of fluoride-bearing minerals. Alkalinity mobilizes fluoride from fluorite with precipitation of calcium carbonate, because the solubility of CaF_2 increases with an increase in $NaHCO_3$ (Handa 1975; Saxena and Ahmed 2001).

$$CaF_2 + 2NaHCO_3 = CaCO_3 + 2Na + \ +2F^- + H_2O + CO_2$$

The above equation clearly shows the processes that could control negative (between fluoride and calcium) and positive relationships (between fluoride and bicarbonate) when both are in contact with each other. Water samples in which fluoride levels exceed 5 mg/L are oversaturated with regard to fluorite. Once fluorite reaches equilibrium, calcite is removed by precipitation, which allows the fluoride concentration to increase (Kim and Young Jeong 2005). In groundwater, the natural concentration of fluoride depends on the geologic, chemical, and physical characteristics of the aquifers, porosity and the acidity of the soils and rocks, the temperature, the action of other chemical elements, and the depth of the wells. In natural water, the fluoride forms strong complexes with Al, and therefore, fluorine chemistry is largely regulated by Al concentration and pH level (Skjelkvale 1994). Below pH 5, fluoride is almost entirely complexed with Al, predominantly with the $AlF_2{}^+$ complex, and consequently the concentration of free fluoride is reduced to low levels. As the pH increases, the Al–OH complexes dominate over the Al–F complexes, and the free fluoride level increases. Fluoride occurs at some level in almost all groundwater, but the concentration found in most potable waters is less than 1 mg/L (Hem 1985).

It has been postulated that fluoride-bearing minerals are normally only sparingly water soluble, with the exception of villiaumite, and these minerals release fluoride to water slowly (Cronin et al. 2000; Saxena and Ahmed 2003). The rate of fluorite dissolution may be faster in sodium bicarbonate-containing waters, and the release of fluoride from clay minerals depends strongly on the pH level (Apambire et al. 1997; Saxena and Ahmed 2003). The maximum concentration of fluoride in groundwater is usually controlled by the solubility of fluorite (Handa 1975; Apambire et al. 1997; Cronin et al. 2000; Saxena and Ahmed 2003; Chae et al. 2007). Once the solubility limit for fluorite (CaF_2) is reached, an inverse relationship will exist between fluoride and calcium concentrations (Fig. 3). Earlier studies have revealed that there is a close association between high fluoride content and soft, alkaline (i.e., sodium bicarbonate) groundwater that is depleted of calcium (Handa 1975; Whittemore et al. 1993; Bardsen et al. 1996; Gupta et al. 1999; Chae et al. 2007). Igneous rocks that have been formed from highly evolved magmas are a rich source of fluorine-bearing minerals. The plagioclase composition of igneous rocks is typically high in albite, the sodium-rich endmember (Hyndman 1985). As a result, the groundwater in contact with these rocks is often soft and calcium deficient, which allows for higher fluoride concentrations when equilibrium with fluorite is attained (Ozsvath 2006).

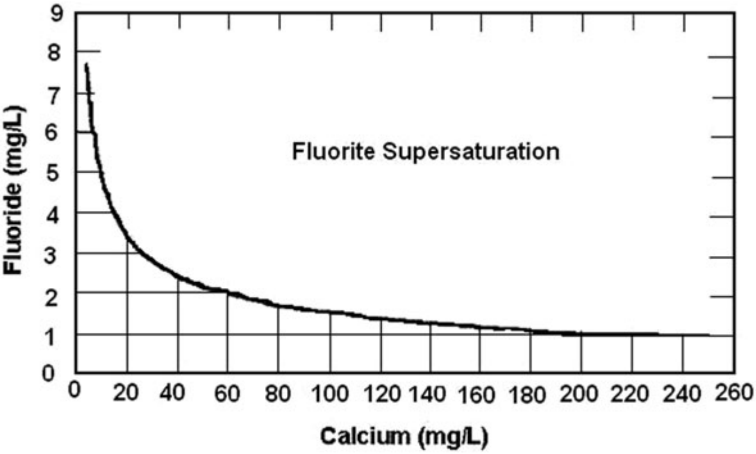


Fig. 3 Fluorite (CaF_2) water solubility curve at 10°C, ignoring the effects of ionic strength and complexes on mineral solubility

High fluoride concentrations (up to 30 mg/L) can also result from anion exchange (OH^- for F^-) with certain clay minerals, weathered mica, and oxyhydroxides that are typically found in residual soils and sedimentary deposits (Whittemore et al. 1993; Apambire et al. 1997; Warren et al. 2005). Laboratory studies have shown that aluminum hydroxides have an especially high fluoride exchange capacity (Cronin et al. 2000). It has been found in past research that a direct relationship exists between pH levels and fluoride concentrations. In some cases, the influence of residence time produces a direct relationship between fluoride concentrations and the depth at which a water sample was collected (Hudak and Sanmanee 2003; Edmunds and Smedley 2005; Kim and Jeong 2005; Chae et al. 2007). The influence of climate on fluoride concentrations in groundwater is largely attributed to rainfall, and to recharge rates and groundwater flow (Edmunds and Smedley 2005). Areas of high rainfall, such as humid tropical regions, are less likely to have high fluoride concentrations in groundwater, because soluble ions such as fluoride are leached out and diluted. Conversely, some arid environments are noted for having high fluoride content, because the low rates of groundwater recharge lead to prolonged water–mineral interaction and higher salinities; such recharge enhances mineral dissolution (Handa 1975; Smedley et al. 2002). Climate can also influence dissolved fluoride levels. Temperature has a direct effect on the solubility of fluorine-bearing minerals. For example, the equilibrium constant for fluorite increases from $10^{-10.80}$ at 10°C to $10^{-10.57}$ at 25°C (Edmunds and Smedley 2005), which allows for roughly 30% more fluoride to dissolve in dilute solutions (Table 2).

It is estimated that approximately 200 million people, representing 25 nations worldwide, are sufficiently exposed to F to put them at risk of having fluorosis. High fluoride levels have been reported to exist in groundwater samples taken from large parts of Africa, China, the Middle East, and southern Asia (India and Sri Lanka).

Table 2 Effect of temperature on fluorite solubility[a]

Calcium conc. (mg/L)	Fluoride concentration at equilibrium with fluorite (mg/L) At 10°C	At 25°C
4	7.56	9.86
8	5.35	6.97
16	3.78	4.93
32	2.67	3.49
64	1.89	2.46
128	1.34	1.74
256	0.95	1.23

Source: Edmunds and Smedley (2005)

[a] Calculations are based on a K_{sp} of $10^{-10.57}$ at 25°C and $10^{-10.80}$ at 10°C, ignoring the effects of ionic strength and complexes on mineral solubility

One of the best known high fluoride belts on land extends along the East African Rift from Eritrea to Malawi, and along a belt from Turkey through Iraq, Iran, Afghanistan, India, northern Thailand, and China. The Americas and Japan have similar belts (WHO 2005).

The intensity of the fluorosis problem has been reported in populous countries such as India and China (Cao et al. 1997; Fung et al. 1999; Mekonen et al. 2001). In 1995, one-tenth of the population of China was exposed to endemic fluorosis (Wang and Huang 1995). The elevated fluoride level in drinking water in China resulted in more than 26 million people suffering from dental fluorosis, and an additional one million people suffering from skeletal fluorosis, in 2004. At Yellowstone National Park in the United States, the amount of fluoride found in hot springs ranged from 25 to 50 ppm (Neuhold and Sigler 1960). In Mexico, five million people (about 6% of the population) are affected by consuming fluoride-contaminated groundwater (UNICEF 1999). Poland, Finland, and the Czech Republic have levels of fluoride in drinking water as high as 3 ppm (Czarnowski et al. 1996; Lahermo et al. 1990). In the Ethiopian Rift Valley, fluoride concentrations in the range of 1.5–177 ppm have been reported (Kloos et al. 1993). Other badly affected areas include the arid parts of northern China (Inner Mongolia), African countries like Ivory Coast, Senegal, North Algeria, Uganda, Ethiopia, northern Mexico, and central Argentina (WHO 2005). In addition, Mambali (1982) reported the incidence of fluorosis associated with excess fluoride intake from groundwater in several African countries (Tanzania, South Africa, Kenya, Korea, Ghana, Sudan, and Tanzania); these countries represent areas of the Rift Valley known to be among the most severely fluoride-affected countries in the world. In many fluorotic areas, people, particularly children, face mobility difficulties from crippling skeletal fluorosis (Mjengera and Mkongo 2003). India also faces this same problem.

In India, fluorosis was first detected by Short et al. (1937) in the Prakasam District, Andhra Pradesh. At that time the disease was prevalent in only four states, namely Andhra Pradesh, Tamil Nadu, Punjab, and Uttar Pradesh. Fluorosis in 1999 was known to be endemic in 17 Indian states (UNICEF 1999). It has been estimated that 62 million Indian people, including 6 million children, suffer from fluorosis that results from consuming fluoride-contaminated water (Susheela et al. 1993). In India, the Bureau of Indian Standards has prescribed the limit of fluoride in potable water to be between 1.0 and 1.5 mg/L (BIS 1991).

6 Fluoride Metabolism in Humans

The amount of fluoride that a person breathes in daily is much less than the amount that is taken in when consumed through food and water. The reason for this is that air fluoride levels are usually less than 1.0 $\mu g/m^3$. The highest natural water level of fluoride reported was 2800 ppm (WHO 2004). In India, concentrations of 0.1–0.3 ppm of fluoride are reported in rainwater (Das et al. 1981; Singh et al. 2001). Surface water concentrations generally range from 0.01 to 0.3 ppm, whereas seawater contains a higher fluoride concentration that is in the range of 1.2–1.5 ppm (WHO 2002).

6.1 Absorption of Fluoride

In humans, the dominating route of fluoride absorption is via the gastrointestinal tract. Airborne fluoride may also be inhaled. Fluoride ions are released from readily soluble compounds such as sodium fluoride, hydrogen fluoride, fluorosilicic acid, and sodium monofluorophosphate, and are almost completely absorbed. The mechanism of F absorption is by passive diffusion. There is no convincing evidence that active transport processes are involved. Fluoride is absorbed into the general circulation from both the stomach and the intestine. Fluoride is absorbed in the form of the undissociated weak acid hydrogen fluoride, HF, which has a pK_a value of 3.45 [i.e., when ionic fluoride enters the acidic environment of the stomach lumen, it is largely converted into HF (Whitford and Pashley 1984)], and up to 40% of HF is absorbed in the stomach and additional amounts are absorbed in the intestine. According to Whitford (1997), 75–90% of the ingested fluoride is absorbed. Once absorbed into the bloodstream, the fluoride is readily distributed throughout the body; approximately 99% of the body burden of fluoride is retained in calcium-rich areas, such as bones and teeth (i.e., dentine and enamels) (WHO 1997). However, in plasma, fluoride is transported as ionic fluoride and non-ionic fluoride. Ionic fluoride does not bind to plasma proteins and is easily excreted via urine. When fluoride is in the form of HF, about 35–45% is reabsorbed and returned to the systematic circulation. The pH of tubular fluid and urinary flow are the main factors that influence the degree of reabsorption (Whitford et al. 1976).

6.2 Bioavailability

Fluoride compounds that either are added or occur naturally in drinking water yield fluoride ion (F^-), which, as stated, is almost completely absorbed from the gastrointestinal tract. Thus, fluoride in drinking water is generally bioavailable. There is only limited information available on the bioavailability of fluoride from fluoride-containing diets. Ekstrand et al. (1994) found that the bioavailability of fluoride in the infant diet was almost 90%. The ingestion of fluoride with food retards absorption and reduces bioavailability. When fluoride was ingested as sodium fluoride tablets on a fasting stomach, the bioavailability of fluoride was almost 100%. When the same dose was taken with a glass of milk, the bioavailability decreased to 70%. When it was taken with a calcium-rich breakfast, the bioavailability was further reduced to 60% (Ekstrand and Ehrnebo 1979; Shulman and Vallejo 1990). The decrease in absorption associated with the ingestion of milk or food probably results from the binding of fluoride with certain food constituents, including calcium and other divalent and trivalent cations. When this occurs, the fecal excretion of fluoride will increase. The concentration of fluoride in soft tissues is reflected by the amounts that appear in blood. Fluoride is concentrated to high levels within kidney tubules and has a higher concentration than does plasma (WHO 2002). Therefore, the kidney could be a potential site and target for chronic fluoride toxicity as a result of its exposure to relatively high fluoride concentrations (NRC 1993). In humans, the placenta regulates the transfer of fluoride from maternal to fetal blood (Gedalia 1970), but fluoride is only poorly transported from plasma to milk (Ekstrand et al. 1984). In human milk, fluoride levels have been measured to be between 5 and 10 μg/L (Fomon and Ekstrand 1999).

The timing of fluoride ingestion affects fluoride bioavailability. When a few grams of fluoride dentifrice are swallowed on a fasting stomach, the plasma peak is recorded within 30 min. However, when the dentifrices are swallowed 15 min after a meal, the peak does not occur until after 1 h (Ekstrand 1987). In the case of gaseous and particulate fluorides, there is partial to complete absorption from the respiratory tract (McIvor 1990), with the extent of absorption being dependent upon solubility and particle size. Particulate fluoride that is deposited in the bronchioles and nasopharynx may be swallowed (via ciliary clearance and/or coughing) and may thus be absorbed via the gastrointestinal tract.

7 Health Effects of Fluoride Exposure

Fluoride intake is often regarded as a double-edged sword. When consumed in inadequate quantities (less than 0.5 ppm), F causes health problems (e.g., dental caries, lack of formation of dental enamel, and deficiency of mineralization of bones), especially among children (WHO 1996). In contrast, if fluoride is consumed or used in excess (more than 1.0 ppm), it can cause health problems in the young, old, or both (WHO 1996). The various forms of fluorosis that may arise from excessive intake of fluoride through drinking water are summarized in Table 3.

Table 3 Effects of fluoride ingestion on human health

Fluoride concentration (mg/L)	Effects
< 0.5	Conducive to dental caries
0.5–1.5	Promotes development of strong bones and teeth
1.5–4.0	Promotes dental fluorosis in children
> 4.0	Promotes dental and skeletal fluorosis
> 10	Crippling skeletal fluorosis, possibly cancer

Source: Dissanayake (1991)

7.1 Dental Fluorosis

Dental fluorosis, which is characterized by mottling of tooth surfaces or enamel, is the condition which first led to the discovery that a relationship exists between fluoride ingestion and human health. As enamel develops, there is an increased mineralization within the developing tooth accompanied by the loss of matrix proteins. Exposure to fluoride during this process causes a dose-related disruption of enamel mineralization, resulting in anomalously large gaps in its crystalline structure, excessive retention of enamel proteins, and increased porosity (Aoba and Fejerskov 2002). Fluoride at excessive intake levels causes the enamel to lose its luster. In its mild form, dental fluorosis is characterized by the appearance of white, opaque areas on the tooth surface, and in severe form, it is manifested by the appearance of yellowish brown to black stains and severe pitting of the teeth. This discoloration may be in the form of spots or horizontal streaks (Choubisa et al. 1996). Normally, the degree of dental fluorosis depends on the amount of fluoride exposure up to the age of 8–10. This is true because fluoride stains only the developing teeth while they are being formed in the jawbones and are still under the gums. The effect of dental fluorosis may not be apparent if the teeth are already fully grown prior to excessive fluoride exposure.

7.2 Skeletal Fluorosis

Skeletal fluorosis is characterized by increased bone mass and density. It affects both children and adults. It does not easily manifest itself until the disease attains an advanced stage. Fluoride is mainly deposited in the joints of the neck, knee, pelvic, and shoulder bones, and once it takes place, it makes movement or walking difficult. The symptoms of skeletal fluorosis are similar to those of spondylitis or arthritis. Early symptoms include sporadic pain, back stiffness, burning-like sensation, pricking and tingling in the limbs, muscle weakness, chronic fatigue, and abnormal calcium deposits in bones and ligaments. At an advanced stage, osteoporosis in long bones and bony outgrowths may occur. Vertebrae may fuse together and eventually become a continuous column of bone. A rare bone cancer, osteosarcoma, may result, and finally, the spine, major joints, muscles, and the nervous system may sustain damage. Crippling skeletal fluorosis is the advanced and severe form of skeletal fluorosis. The prevalence of high levels of fluoride intake over the long term, accompanied by malnutrition, strenuous manual labor, and impaired renal function, leads to severe skeletal fluorosis (Reddy 1985). Usually men suffer higher rates of severe fluorosis than do women, presumably because they more often do strenuous work (Jolly et al. 1968; Siddiqui 1955). "Genu valgum" (knock knees) and "Genu varum" (bow legs) are two important forms of skeletal fluorosis that are observed in India. The extremely severe form of fluorosis, "Genu valgum," has been observed with osteosclerosis of the spine and concomitant osteoporosis of the limb bones, and with very high serum parathyroid hormone levels suggestive of hyperparathyroidism (Krishnamachari and Krishnaswamy 1973). Patients suffering from fluorosis usually experience difficulty in walking because of the development of progressive weakness in the lower limbs. The receptivity of fluoride on the skeletal surface is a function of bone age and type, with young and cancellous bone being more receptive than old or cortical bone (WHO 1970). The concentration of fluoride in the bone also varies with age, sex, type, and specific part of bone, and is believed to reflect an individual's long-term exposure to fluoride (WHO 2002). It was observed that approximately 99% of the fluoride in the body is found in bones and teeth (Hamilton 1990; Kaminsky et al. 1990) (though the amount of fluoride in teeth is very small compared to bones), with the remainder distributed in highly vascularized soft tissues and blood.

Besides skeletal and dental fluorosis, excessive consumption of fluoride may lead to many other disease manifestations: neurological manifestations, depression, gastrointestinal problems, urinary tract malfunctioning, nausea, abdominal pain, tingling sensation in fingers and toes, muscle fiber degeneration, low hemoglobin levels, deformities in RBCs, excessive thirst, headache, skin rashes, nervousness, reduced immunity, repeated abortions or still births, male sterility, reduced intelligence, etc. Jacobsen et al. (1992) also found a significantly elevated risk of hip fractures in residents living in countries with fluoridated water.

7.3 Exposure and Safety Guidelines

As given by the World Health Organization (WHO 1997), the maximum limits of fluoride in drinking water should be 1.5 mg/L. However, the US Public Health Service (USPHS 1962) has set a range of allowable concentrations for fluoride in drinking water that are based on climatic conditions, because the amount of water consumed and the amount of fluoride ingested is influenced by air temperature. Table 4 shows the maximum allowable fluoride concentration as established by the USPHS. In hot tropical areas of the world, people consume more water and consequently the risk of fluoride accumulation increases. People who inhabit tropical or semi-arid countries that lie between latitudes 10° and 30° north and south of the

Table 4 USPHS[a] recommendations for maximum allowable fluoride in drinking water

	Recommended fluoride conc. (mg/L)			
Annual average maximum daily air temperature (°C)	Lower	Optimum	Upper	Maximum allowable fluoride concentration (mg/L)
10–12	0.9	1.2	1.7	2.4
12.1–14.6	0.8	1.1	1.5	2.2
14.7–17.7	0.8	1.0	1.3	2.0
17.8–21.4	0.7	0.9	1.2	1.8
21.5–26.2	0.7	0.8	1.0	1.6
26.3–32.5	0.6	0.7	0.8	1.4

[a] United States Public Health Service

equator suffer extensively from endemic fluorosis (UNEP-WHO 1992). The inhabitants of the majority of these countries that live in such zones are malnourished and are poor. WHO (1984) guidelines suggest that in areas with a warmer climate the optimal fluoride concentration in drinking water should remain below 1 mg/L; in cooler climates, F levels of up to 1.2 mg/L may exist.

The lethal dose of sodium fluoride for an average human adult has been estimated to be between 5 and 10 g (32–64 mg F/kg body weight) (WHO 1984; Whitford 1990). The tolerable upper intake level of fluorine ranges from 0.7 to 2.2 mg/day for children and 10 mg/day for adult men and women (NRC 1993), whereas the US EPA has set an oral reference dose (RfD) of 0.06 mg/kg/day for fluorine (ATSDR 2003). The maximum amount of fluoride allowed by EPA in drinking water is 4 mg/L.

It has been reported that the average dietary intake of fluoride ranges between 0.02 and 0.048 mg/kg/day for adults living in areas with 1.0 ppm fluoride in the water. In areas with less than 0.3 ppm fluoride in water, the adult dietary intake ranges from 0.004 to 0.014 mg/kg/day. In children, the dietary intake ranges from 0.03 to 0.06 mg/kg/day in areas with fluoridated water and is 0.01–0.04 mg/kg/day in areas without fluoridated water (ATSDR 2003). Jha et al. (2009) determined the exposure levels of fluoride in infants (6 kg body weight), children (20 kg body weight), and adults (70 kg body weight) that were found in drinking water sourced from shallow aquifers; the levels found ranged from 0.36 to 4.63, 0.162 to 2.08, and 0.092 to 1.19 mg/kg/day, respectively. Infants, bottle-fed on milk formula that has been reconstituted with fluoridated water, ingest F at levels that range from 0.12 to 0.18 mg/kg/day. For infants of a typical mass (8.1 kg at age 6 months), this gives total fluoride intakes of 1.0–1.5 mg/day, which are equivalent to adult doses (Diesendorf and Diesendorf 1997). Li et al. (2001) suggested a no-observed-adverse-effect level (NOAEL) to be 0.15 mg fluoride/kg/day and a lowest observed-adverse-effect level (LOAEL) to be 0.25 mg fluoride/kg/day in humans. However, these values must be evaluated in the context of the local situation and may be different in different locations. Because different researchers or agencies have stipulated different guidelines for fluoride exposure, any evaluation of safe

F exposure should incorporate systematic epidemiological surveillance for dental and skeletal fluorosis in the fluoride-affected areas. Otherwise, it will be difficult to understand the local-specific human dose–response relationship.

8 Summary

The presence of environmental fluoride and its impact on human health is well documented. When consumed in adequate quantity, fluoride prevents dental caries, assists in the formation of dental enamels, and prevents deficiencies in bone mineralization. At excessive exposure levels, ingestion of fluoride causes dental fluorosis, skeletal fluorosis, and manifestations such as gastrointestinal, neurological, and urinary problems. The distribution of fluoride in the environment is uneven and largely is believed to derive from geogenic causes. The natural sources of fluoride are fluorite, fluorapatite, and cryolite, whereas anthropogenic sources include coal burning, oil refining, steel production, brick-making industries, and phosphatic fertilizer plants, among others. Among the various sources of fluoride in the environment, those of anthropogenic origin have occasionally been considered to be major ones. The groundwater is more susceptible to fluoride accumulation and contamination than are other environmental media, primarily because of its contact with geological substrates underneath. The high fluoride concentration in water usually reflects the solubility of fluorite (CaF_2). High concentrations are also often associated with soft, alkaline, and calcium-deficient waters. The fluoride compounds that occur naturally in drinking water are almost totally bioavailable (90%) and are completely absorbed from the gastrointestinal tract. As a result, drinking water is considered to be the potential source of fluoride that causes fluorosis. Because the bioavailability of fluoride is generally reduced in humans when consumed with milk or a calcium-rich diet, it is highly recommended that the inhabitants of fluoride-contaminated areas should incorporate calcium-rich foods in their routine diet. Guidelines for limiting the fluoride intake from drinking water have been postulated by various authorities. Such limits are designed to protect public health and should reflect all fluoride intake sources, including dietary fluoride. The toxicological risks posed by fluoride could be better understood if epidemiological surveillance for dental and skeletal fluorosis would be systematically conducted in fluoride-affected areas. Such input would greatly improve understanding of the human dose–response relationship. Such surveillance of potentially high fluoride areas is also important because it would help to delineate, much earlier, the remedial measures that are appropriate for those areas.

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