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David M. Whitacre *Editor*

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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 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 facing 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 everincreasing 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, NC David M. Whitacre

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Evaluating Risks of Acquired Clinical Vulnerability Among Subjects Exposed to E-Waste

Anup Kumar Srivastava, Chandrasekharan Nair Kesavachandran, and Sushil Kumar

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1 Introduction

Long-term exposures to some environmental toxicants are known to induce chronic diseases. Certain diseases, such as lung cancer, may result from chronic exposures that are lower than those caused by certain "classical" carcinogens (e.g., tobacco smoke) (Vineis et al. 2004). Diseases result from antecedent events (Rothman and Greenland 1998). Such events either complete an incomplete causal chain that allow disease onset (Vineis and Kriebel 2006) or precipitate a chain of events, which creates a clinical state of vulnerability. Clinical vulnerability may render exposed subjects more susceptible to morbidity(s) following low dose long-term exposure to some environmental toxicants. When increased vulnerability occurs, it may either be acquired or be of genetic origin. The concept of acquired clinical vulnerability (ACV) results from insults that produce consequential pathophysiological changes

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that predispose exposed subjects to disease. ACV comprises a complex biological process that is manifested by exposure to toxicants, generally over the course of many years, and results from subtle changes that occur at the cellular and molecular level.

In this paper, we review the available literature on health-hazard threats posed by the handling of electronic waste (E-waste), predominantly in third-world countries, and evaluate the resulting exposures in the context of ACV.

2 Electronic Waste

E-waste comprises a multitude of electronic components of virtually any electronic device that has become unusable (Tables 1 and 2). Most E-waste results from the fact that electronic devices age rapidly (technologically), are relatively inexpensive, and are replaced by newer and usually more powerful devices that serve the same function. In India, an enormous magnitude of E-waste generation is expected in the next decade and beyond. This increase will result from plans to escalate India's economic growth to achieve a target of 11% share of the global consumer electronic market by 2015. According to conservative estimates, there will be 600 million mobile subscribers, 60 million Personal Computers (PCs), and 143 million TVs in India by the year 2012. Most of the electronic gadgets owned by the population contain economically valuable materials. Nevertheless, more disconcerting is the fact that most, if not all, of these items will contain materials that are unequivocally dangerous to the environment and/or to human health, if processed for recycling, disposed of in landfills, or are incinerated. It is rather certain that some proportion

Table 1 Material composition (% by weight) of E-waste from selected appliances

Material	Large household appliances	Small household appliances	Information and communication technology (ICT) and consumer electronics	Lamps
Ferrous metal	43	29	36	–
Aluminum	14	9.3	5	14
Copper	12	17	4	0.22
Lead	1.6	0.57	0.29	–
Cadmium	0.0014	0.0068	0.018	–
Mercury	0.000038	0.000018	0.00007	0.02
Gold	0.00000067	0.00000061	0.00024	–
Silver	0.0000077	0.000007	0.0012	–
Palladium	0.0000003	0.00000024	0.00006	–
Indium	–	–	0.0005	0.0005
Brominated plastics	0.29	0.75	18	3.7
Plastics	19	37	12	–
Lead glass	–	–	19	–
Glass	0.017	0.16	0.3	77
Other	10	6.9	5.7	5

Source: E-waste guide information (2010)

Table 2 Composition of selected appliance E-waste: absolute and percent composition of various components

Appliance type	Refrigerators and freezers	TV sets
Average weight (kg)	48	36.2
Iron (%) (used for casings and frames)	64.4	5.3
Non ferrous metal (%) (copper used in cables and aluminum)	6	5.4
Glass (%) (used for screens)	1.4	62
Others (%) (rubber, wood, ceramic, etc.)	15.1	3.5

Source: Sahu and Agarwal (2008)

of E-waste is, and will be, disposed of by all the three methods. Certainly, there is value in recycling of E-waste. E-waste recycling revenues in India have the potential to reach a value of US$ 350 million annually by the year 2012 (Tata Strategic Management group 2010).

Workers in the E-waste disposal sector are poorly protected against the risks to which they are exposed. These workers dismantle a multitude of different electronic gadgets, often by hand, under appalling conditions. Approximately 25,000 workers are employed at scrap-yards in Delhi alone. In these E-waste scrap-yards, 10,000–20,000 tons of E-waste is handled every year, with computers accounting for 25% of the total. Other E-waste scrap-yards exist in Meerut, Firozabad, Chennai, Bangalore, and Mumbai (Pinto 2008). In addition to those who work in legitimate E-waste scrap-yards, a large number of other people become exposed to contaminants related to E-waste. Among these are subjects who reside in the areas adjacent to where E-waste is either processed or disposed of, i.e., landfills, incineration sites, and illegal scrap processing units. It is difficult to estimate how many such sites actually exist, because many are clandestine; however, they have pervaded nearly all major cities in this country (Pinto 2008). In addition, E-waste disposal in India assumes greater significance, not only because of the amounts of locally generated E-waste but also from "dumping" of E-waste (particularly computer waste) from countries that did not ratify the terms of the Basel Convention.

2.1 E-Waste Generation and Regulation

The E-waste trade is an emerging and increasingly prominent business that occurs primarily in developing countries such as India, where goods recycling are widely performed. Participants in the E-waste business generally constitute small operations that are informally organized and are not registered as legitimate enterprises. These E-waste traders often collect the E-waste from impoverished scrap-collectors, who carry their items on bicycles and utilize the cheapest way to recycle that they can find. This sector seldom follows safe or environment-friendly practices or guidelines because such practices may add to their costs, and one of their primary

goals is to maximize profits. Notwithstanding the foregoing statements, there are several legitimate companies in India that recycle E-waste. Among these legitimate enterprises are Info Trek, Trishyiraya, e-Parisara, Ultrust Solutions, and Ramky.

India generates about 150,000 tons of E-waste annually. Almost all of this E-waste presently ends up in the hands of those who work in the informal sector, because organized alternatives are not available (TERI 2006). Metropolitan Indian cities, such as Delhi, Mumbai, and Bangalore, have centers where E-wastes are stockpiled. Such stockpiles have risks of their own. According to a study conducted by Toxic Links (Pandve 2007), Mumbai City generates 19,000 ton of E-waste, which is incremental to the large amounts that are clandestinely imported. The large volume of E-waste generation, coupled with uncontrolled disposal practices, pose grave environmental and health risks to Mumbai City, because of its dense population and spatial character (Urban Hazards 2010). The Karnataka State Pollution Control Board admits to the generation of 10,000 ton/month of E-waste, chiefly in Bangalore. The risk such waste and its disposal has to the environment and to public health in the Indian Silicon city has been noted (Shy et al. 2009).

The clandestine import of E-waste to developing countries has greatly aggravated the E-waste exposure problem. The E-waste trade is controlled by the Basel Convention (Electronic Waste 2009). However, E-waste is sent for processing, sometimes illegally, to several countries: China, Malaysia, India, Kenya, and other African countries. Examples of important E-waste processing areas are Guiyu in the Shantou region of China, and Delhi and Bangalore in India (Nnorom and Osibanjo 2008). There are several reasons as to why these countries are selected by exporters, including the following: they have inadequate environmental or industry–labor relations standards, cheap labor, and relatively high profit margins for recovered raw materials for those involved in E-waste trading. Because the USA neither has ratified the Basel Convention nor has domestic laws forbidding the export of toxic E-waste, a large part of its E-waste is directed overseas for recycling, according to estimates of the Basel Action Network (Electronic Waste 2009). The Basel Convention (formally called the Basel Convention on the Control of Transboundary Movements of Hazardous Wastes) is an international treaty designed to reduce the movement of hazardous E-waste between nations, and specifically to prevent transfer of hazardous waste from developed to less developed countries (LDCs). The Basel Convention specifically excluded the movement of radioactive waste (Electronic Waste 2009). The Convention terms are intended to do the following:

- Minimize the amount and toxicity of wastes generated
- Ensure that environmentally safe management practices are followed in LDCs similar to those that exist at the source of generation
- Assist LDCs to implement environmentally safe management practices for handling the hazardous and other wastes they generate

Because of the dearth of governmental legislation on E-waste, lack of standards for its disposal, and proper mechanisms of handling, most toxic E-waste ends up in landfills. Moreover, E-waste that has only been partly recycled is often stored, transported or handled under unhygienic conditions. However, times are changing, since

Table 3 Composition of materials present in a typical personal computer

Elements	Percentage of total weight	Content (g)	Elements	Percentage of total weight	Content (g)
Plastics	23	6,250	Zinc	2	55
Lead	6	1,710	Beryllium	0.0157	4.26
Aluminum	14	3,850	Gold	0.0016	0.434
Germanium	0.0016	0.4345	Cobalt	0.0157	4.2
Gallium	0.0013	0.3531	Palladium	0.0003	0.081
Iron	20	5,570	Manganese	0.0315	8.55
Tin	1	271	Silver	0.0189	5.13
Copper	7	1,880	Mercury	0.0022	0.597
Barium	0.0315	8.55	Arsenic	0.0013	0.35
Nickel	0.8503	231	Silica	24.8803	6.770

Source: Sahu and Agarwal (2008)

E-waste management and handling rules were implemented in 2010 by India's Ministry of Environment and Forests and will come into force by January 1, 2012. These draft rules include the following provisions:

- They impose responsibilities on producers, distributors, refurbishers, collection centers, consumers, dismantlers, recyclers, and reprocessors
- They include authorization and registration procedures for handling E- waste
- Among other restrictions they designate procedures for storing E-waste
- They reduce the use of hazardous substances (RoHS) in the manufacture of electrical and electronic equipment (MOEF 2010)

2.2 Composition of E-Waste

Some of the more toxic substances that are found in E-waste include lead, mercury, and human carcinogens, such as cadmium, and the polychlorinated biphenyls (PCBs). A typical computer monitor may contain more than 6% lead by weight, much of which is in the lead glass of the Cathode Ray Tube (CRT). The composition of materials present in a typical personal computer is shown in Table 3. Capacitors, transformers, polyvinylchloride (PVC)-insulated wires, and PVC-coated components, manufactured before 1977, often contain dangerous levels of PCBs (Zabrosky 2010).

On the average, when one ton of E-waste is shredded and undergoes the standard separation steps that comprise mechanical recycling, approximately 40 kg of a precious-metal containing dust-like material is generated. When present in this concentrated form, this material can be toxic to exposed individuals (Gupta et al. 2008). Other substances in E-waste that are found in large quantities include epoxy resins, fiber glass, PCBs, PVC, lead, tin, copper, silicon, beryllium, carbon, iron, and aluminum, as well as thermosetting plastics. Elements that are found in small amounts include cadmium, mercury, and thallium (Electronic Waste 2009). In addition, certain

other elements are found in E-waste, but only in trace amounts. These include americium, antimony, arsenic, barium, bismuth, boron, cobalt, europium, gallium, germanium, gold, indium, lithium, manganese, nickel, niobium, palladium, platinum, rhodium, ruthenium, selenium, silver, tantalum, terbium, thorium, titanium, vanadium, and yttrium (Electronic Waste 2009).

2.3 E-Waste Recycling in India

There are five legitimate recycling units in India. These are located at Mumbai, Bangalore, and Chennai. However, there are illegitimate E-waste scrap-yards, and the cities that host major ones are Meerut, Firozabad, Chennai, Bangalore, and Mumbai (Pinto 2008). Workers that are involved in the E-waste recycling are known to use rather crude methods and are directly exposed to many chemicals as they perform their work. The types of operations that are involved in illegitimate recycling of E-waste include e-material collection, sorting, transportation, and dismantling. In addition, illegitimate workers may be involved in separation and recovery of usable parts or precious metals (by acid leaching techniques) and open-air incineration of unusable parts.

However, nonworkers also become exposed because they reside in areas adjacent to locations in which E-waste is either disposed of, or is processed. Such sites include landfills, incineration sites, and illegal scrap processing operations. It is not known how many such illegal E-waste processing operations exist, but it is known that they pervade nearly all major cities in India (Pinto 2008).

3 Hazards to Human Health

In Table 4, we summarize the health effects in humans that may result from exposure to the chemical constituents in E-waste. The short-term clinical symptoms resulting from E-waste exposure have been profiled; however, the problem of chronic-exposure to low doses of multiple chemicals and the associated pathophysiology (clinical vulnerability) associated with E-waste-exposed subjects remains unaddressed.

There is a paucity of reports on the toxicoepidemiology of the subclinical effects that result from E-waste exposure. A single citation exists from Guiyu, a town in the Guangdong province of southern China. In Guiyu, both imported and domestic E-waste is processed to extract valuable metals for sale or reuse. Huo et al. (2007) compared the blood lead levels (BLLs) and blood cadmium levels (BCLs) of 154 children that were less than 8 years of age from Guiyu, with 124 children of the same age from Chendian, a town that had no E-waste processing industry. The proportion of the children in Guiyu that had BLLs indicative of lead poisoning (70.8%) was far higher than those of Chendian children (38.7%). BCLs of Guiyu children were also significantly higher (20.1%) as compared to the 7.3% incidence for Chendian children.

Table 4 Effects of toxic E-waste constituents on human health

Sources of E-waste	Toxic constituents present	Health effects	References
Solder in printed circuit boards, glass panels, and gaskets in computer monitors	Lead (Pb)	Damage to central and peripheral nervous systems, blood systems and kidney damage Affects brain development of children	Zheng et al. (2008), Pandve (2007), Pinto (2008), Shy et al. (2009)
Chip resistors and semiconductors	Cadmium (Cd)	Toxic irreversible effects on human health Accumulates in kidney and liver Causes neural damage Teratogenic	Zheng et al. (2008), Pandve (2007), Pinto (2008), Shy et al. (2009)
Relays and switches, printed circuit boards	Mercury (Hg)	Chronic damage to the brain Respiratory and skin disorders due to bioaccumulation in fishes	Shy et al. (2009), Pandve (2007), Pinto (2008)
Corrosion protection of untreated and galvanized steel plates, decorator or hardener for steel housings	Hexavalent chromium (Cr VI)	Asthmatic bronchitis DNA damage	Shy et al. (2009), Pandve (2007)
Cabling and computer housings	Plastics including PVC	Burning produces dioxin that causes reproductive and developmental problems Immune system damage Interfere with regulatory hormones	Shy et al. (2009), Pandve (2007)
Plastic housings of electronic equipment and circuit boards	Brominated flame retardants (BFR)	Disrupts endocrine system function	Shy et al. (2009)
Front panels of CRTs	Barium (Ba)	Short-term exposure causes: Muscle weakness Damage to heart, liver, and spleen	Shy et al. (2009)
Motherboards	Beryllium (Be)	Carcinogenic (lung cancer) Inhalation of fumes and dust causes chronic beryllium disease or beryllicosis Skin diseases such as warts	Shy et al. (2009), Pandve (2007)

In a separate study, the same team established a control group using data from pregnant women in suburban areas of Xiamen, Fujian Province, located 200 km away from Guiyu. This control site (Xiamen, Fujian Province) had no E-waste processing activities. The authors found that, between 2003 and 2007, the rate of premature delivery, fetus-death and low birth-weight in Guiyu were all significantly higher than in the control group. The fetal death rate in Guiyu was approximately six times higher than the rate in the control group, whereas premature delivery was approximately 62% higher.

Processing of E-waste is profitable and continues to attract new workers to the trade; this occurs despite the health risks involved. In China, processing one ton of E-waste yields approximately 450 g of gold and 200 kg of lead (Zheng et al. 2008; Wong et al. 2007). A similar situation exists in India. The importance of E-waste recycling has been highlighted in an occupational health context by Pinto (2008) and Pandve (2007). A study by the Chittaranjan National Cancer Institute at Kolkata found that people in Delhi were twice as likely to suffer from lung ailments as those who reside in the countryside; and the lung ailments that existed were associated with the (huge) amounts of E-waste generated (Toxics Link 2010).

Wen et al. (2008) studied the occupational exposure of workers at two electrical and electronic equipment dismantling factories located in an area of east China. Exposures were monitored to several chemicals, including polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs), polybrominated diphenyl ethers (PBDEs), and PCBs. The monitoring activities addressed indoor dust ($n=3$) in workshops, as well as hair samples from male workers ($n=64$). Pre- and post-work-shift urine samples (64 of each) were also collected from the workers and were analyzed for oxidative damage to DNA, using 8-hydroxy-2′-deoxyguanosine (8-OHdG) as a biomarker. The homologue and congener profiles in the samples demonstrated that high concentrations of PCDD/Fs, PBDEs, and PCBs originated from open-air incineration of E-waste. The 8-OHdG levels were 6.40 ± 1.64 μmol/mol creatinine in pre-work-shift urine samples. However, the levels significantly increased to 24.55 ± 5.96 μmol/mol creatinine in post-work-shift urine samples ($p<0.05$). A high cancer risk was regarded to exist at this E-waste processing site. The risk that originated from the elevated 8-OHdG levels was from the E-waste and was regarded to have caused oxidative stress among the workers involved in E-waste dismantling. Moreover, the oxidative stress that resulted from exposure of workers was believed to result from exposure to the high concentrations of PCDD/Fs, PBDEs, and PCBs present.

Although there are few epidemiology studies that deal directly with the human impact of E-waste, there are other data that strongly suggest increased human disease vulnerability from long-term low-level exposures as does that occurs with E-waste handling.

The authors of this paper believe that concomitant low-dose exposure to a multitude of toxicants over many years leads to subtle changes at the cellular, molecular, and gene-expression levels that are prospectively easy to demonstrate using a genomics approach. Such changes can be examined to determine if there is an association with increased disease vulnerability, viz., cancers, reproductive end

points, neurological problems, and renal damage. In one study, the effects of E-waste toxicants were addressed for a random selection of 429 adolescent and 361 adult subjects (Ketelslegers et al. 2008). These subjects were genotyped for 36 polymorphisms in 23 genes, selected because of their known role in carcinogen metabolism, DNA repair, and oxidative stress. In both age groups, the relationship between endogenous exposure to organochlorines (PCBs, hexachlorobenzene, dichlorodiphenyl dichloroethane), metals (cadmium, lead), and urinary metabolites (1-hydroxypyrene, *trans–trans* muconic acid) vs. genotoxic effects (Comet assay and micronuclei in lymphocytes, and urinary 8-hydroxydeoxyguanosine) was investigated. In adults, the relationship of these exposures was tested using selected tumor markers (prostate-specific antigen, carcinoembryonic antigen, and p 53). The impact of the genotype on established exposure–effect relationships revealed eight exposure–effect relationships, and three novel associations that affected various genotypes, predominantly those involving biotransformation and oxidative stress responses. These techniques may also be applicable to evaluating increased risk of cardiovascular events, metabolic conditions, and alterations in cognitive functions.

3.1 Hazards Associated with Disposal in Landfills

More than 220 papers have been published that address the health hazards associated with landfill sites (Saffron et al. 2003). Of these, 101 were primary studies that concerned the health effects originating from landfill sites, and 23 addressed the health effects of drinking water contaminated with leachate from landfill sites. Six reviews addressed epidemiological evidence linking health effects with landfill sites (Cantor 1997; Miller 1996; Sever 1997; Johnson 1997, Vrijheid 2000). However, the source of contamination that caused human health effects in these studies was not known. In some studies, the source was thought to be leaking chemical storage tanks, and in others, chemical accidents. Study authors that investigated links between landfill sites and communities revealed the plethora of targeted effects that were examined; these included reproductive or developmental effects on children (31 studies), cancer (29 studies), clinical symptoms (28 studies), psychosocial impacts (19 studies), biomarkers (13 studies), health problems not specified (14 studies), mortality (5 studies), and injuries/poisoning (only 2 studies).

The weakness of studies that address the health effects of E-waste at landfills is the complete lack of exposure data. Rather than gathering exposure data at landfill sites, investigators used census data, postal codes, or other information that linked the number of residents having proximity (2–3 km of the site) to the landfill, as a poor proxy for exposure. Because of the lack of exposure data, many studies generated more questions than answers. In addition, none of the hypothesis-testing studies controlled for possible confounding factors. In residential areas, where E-waste facilities sometimes exist, there are many other sources of pollution that make it difficult to assign causality of disease to E-waste-connected activities.

These confounding factors include the following: environmental pollutants emanating from industry or traffic, or concurrent exposure to occupational hazards, indoor air pollutants, tobacco smoke, alcohol, prescription drugs, and recreational drugs. There were more than 20 hypothesis-generating studies, but the results were inconsistent. Some studies showed associations between landfills and various health impacts, while other studies reported no associations. The strongest suggestion for causality was generated by studies having to do with reproductive outcomes, such as reduced birth weight or some birth defects. However, all studies lacked direct exposure assessment, and the limited sample sizes of most studies made a more specific analysis impossible.

Considering all the uncertainties, it is concluded that the data available on landfills and their risks to humans strengthens the suspicion that exposure to emissions from hazardous wastes do pose a risk of ill health to some exposed individuals. However, existing data do not substantiate with sufficient evidence to indicate which landfills, or what exists in them, are the primary culprits that may be responsible for the observed small increases in risk (WHO 1998).

3.2 Hazards Associated with Disposal by Incineration

A search for published reports on incineration of E-waste yielded (Saffron et al. 2003) 50 primary studies and three reviews. The majority were studies on communities, although there were also 14 occupational health studies, in which a wide array of health outcomes were investigated: e.g., cancer (15 studies), health problems/diseases/unspecified health effects (12 studies), biomarkers (10 studies), reproductive outcomes/developmental effects on children (9 studies), symptoms (8 studies), mortality (5 studies), injuries/poisoning (3 studies), psychosocial impacts (2 studies), economic impacts (1 study). Among the occupational health studies, there were three in which exposure was presumed because of worker occupation at the site of the incineration, two studies that utilized quantified ambient measurements of particulate matter (with an aerodynamic diameter ≤10 μm (PM_{10})) or metals, and seven studies that provided quantified personal measurements (of BLLs or of urinary mutagens). Among the studies in which communities had residents living near incinerators, four used quantified ambient measurements, two used quantified estimates, and 27 used residence alone as a proxy for exposure. Furthermore, there were four hypothesis-testing studies (Bresnitz et al. 1992; Shy et al. 1995; Lee and Shy 1999; Gray et al. 1994). Lee and Shy (1999) analyzed how health outcomes varied according to the degree of exposure to ambient pollutants, as well as to other cofactors including, sex, age, respiratory hypersensitivity, period of time spent outdoors within the area of the selected community, and surrogate measures for indoor air pollution exposure (vacuum use and experience of air irritants at work). The four hypothesis-testing studies consistently showed no association between the hazards from incineration and any health outcomes.

4 Conclusions

Human exposure to the array of pollutants that commonly exists in the environment, and the health affects normally recorded as a result of this exposure are tip of the iceberg. The visible clinical manifestations of exposure constitutes only a small fraction of what may exist as latent damage or disease vulnerability, whereas the major portion is submerged in the unknown dimensions of effects that have not been investigated, and hence, are not yet known (Fig. 1). It is this submerged portion that relates to future risks that derive from manifold environmental exposures that predispose to disease susceptibility or clinical vulnerability. The future epidemiological challenge is to better identify, quantify, and evaluate the magnitude of this risk to subjects, who become clinically vulnerable after such exposures.

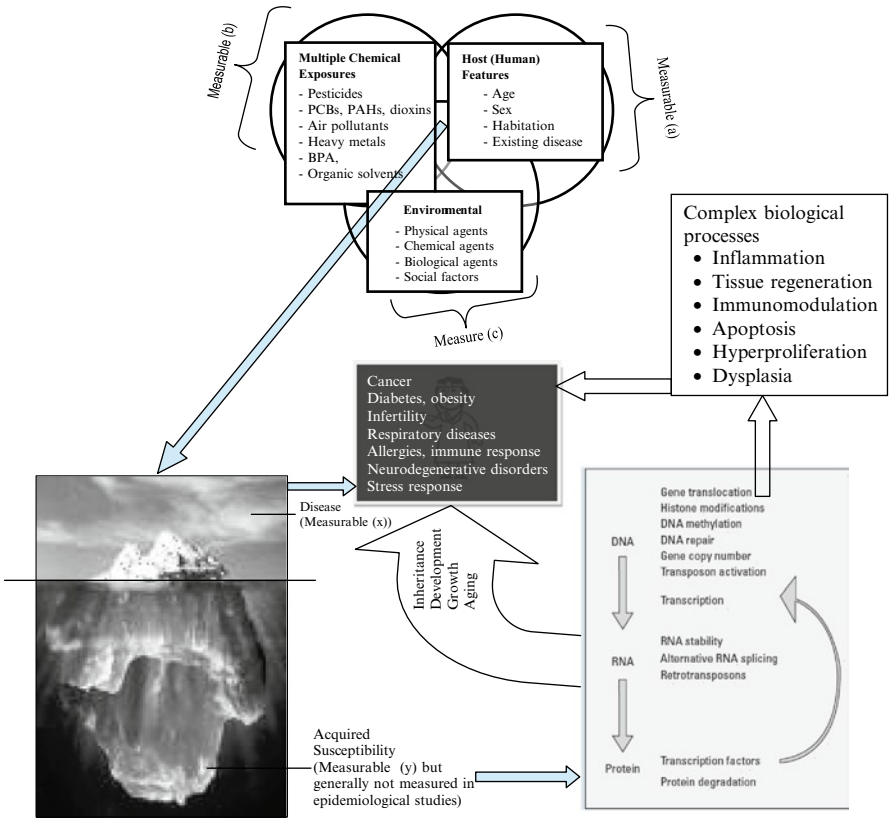

Fig. 1 A diagram of the potential impact of E-waste exposure on humans as it relates to acquired susceptibility. Epidemiologically, a, b, c are input variables and *x*, *y* are output variables. The exact mathematical relationship of a, b, c and *x*, *y* needs to be further explored and quantified. Abbreviations: *PCB* polychlorinated biphenyl, *PAH* polycyclic aromatic hydrocarbons, *BPA* Bisphenol A

A multidisciplinary effort based on environmental epidemiology, holistic exposure assessments, and genomic approaches is required to identify and elucidate the nature and magnitude of such risks. To better identify the hidden risks of multichemical exposure of this sort, development of new biomarkers is required. Conventional methods together with genomic techniques can be innovatively employed to develop biomarkers for early detection of these risks. Prominent targets for such biomarkers may include (1) metabolic pathways that affect cell-signaling, (2) cell adhesions, (3) cytoskeletons, (4) ligand–receptor interactions, etc., which are intermediary in the onset of complex biological responses, viz., inflammation, tissue regeneration, hyperproliferation, dysplasia, immunosuppression, and apoptosis.

Tests based on RT-PCR (Reverse Transcription Polymerase Chain Reaction), and immunoassays, will be tools that can help identify alterations in human genome expression after subclinical effects are caused by chronic low-level exposure to multiple toxicants. Such research endeavors will help to predict acquired susceptibility for the onset of common disorders such as Type-2 diabetes, dyslipidemia, or hypertension that constitute metabolic syndromes that are on rise in Indian subjects (Gupta et al. 2004). The identification of more objectively diagnosed ACV, together with appropriate interventions, may reduce the future burden of E-waste-related diseases.

5 Summary

Acquired clinical vulnerability (ACV) results from insults that produce consequential pathophysiological changes and predispose exposed subjects to future disease. ACV comprises a complex biological process that is manifested by exposure to toxicants, generally over the course of many years, and results from subtle changes that occur at the cellular and molecular level. A large proportion of the world's population has already been, or will be, exposed to toxicants emanating from E-waste during the course of their lives. In countries where E-waste recycling is an important economic activity (China, India, among others), the challenge facing researchers is to devise suitable methods for identifying and objectively measuring ACV. Primary prevention can be achieved through legislation/awareness/monitoring and secondary prevention by developing innovative diagnostic tools and corrective measures.

Studies in which attempts are made to define the health impact of multiple exposures, as routinely occurs in E-waste recycling, should include measures of as many of the following parameters as possible: (a) characterization of pollutant levels in air/water/soil at the residential or workplace, (b) periodical clinical examination of exposed subjects, (c) assessments of circulating toxicant loads in blood/urine/hair, (d) genomic variation and resultant susceptibility to complex biological responses, (viz, inflammation/dysplasia/immunosuppression/tissue regeneration) that derive from pathway modulation (viz., cytoskeleton/metabolism/cell adhesion/immune system/neuroactive ligand–receptor interaction/cytokine/signaling), (e) routine monitoring of altered gene expression from modulation of hematology or the above-mentioned pathways.

E-waste exposure may also serve as a model for the types of multiple exposures that occur in other industrial or environmental exposures. Moreover, the approach used to study and address or alleviate E-waste exposure may also be useful in other environmental exposure situations. The studies necessary to address and alleviate E-waste hazards may eventually be cost-effective, since they are likely to result in manifold savings in reduced health costs, increased human productivity, and reduced indirect social costs.

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References

Bresnitz EA, Roseman J, Becker D, Gracely E (1992) Morbidity among municipal waste incinerator workers. Amer J Ind Med 22;3: 363–78.

Cantor, K P (1997) Drinking water and cancer. Cancer Causes and Control. 8; 3: 292–308.

Electronic Waste (2009) (accessed on 18-11-2009). Available from http://en.wikipedia.org/wiki/Electronic_waste

E waste guide information (2010) Material composition (accessed on 27-5-2010). Available from http:e waste guide.info/material composition.

Gray EJ, Peat JK, Mellis CM, Harrington J, Woocock AJ (1994) Asthma severity and morbidity in a population sample of Sydney school children: part I – prevalence and effect of air pollutants in coastal regions. Austr New Zeal J Med 24: 168–75.

Gupta R, Deedwania PC, Gupta A, Rastogi S, Panwar RB, Kothari K (2004) Prevalence of metabolic syndrome in an Indian urban population, International J Cardiology 97: 257–261.

Gupta V, Laul P, Syal SE. E waste (2008) A waste or a fortune ? Current Science 2008; 94:5:554–555.

Huo X, Peng L, Xu X, Zheng L, Qiu B, Qi Z, Zhang B, Han D, Piao Z (2007) Elevated Blood Lead Levels of Children in Guiyu, an Electronic Waste Recycling Town in China. Environ Hlth Persp 115: 1113–1117.

Johnson BL (1997) Hazardous waste: human health effects. Toxicol Ind Hlth 13; 2–3: 121–43.

Ketelslegers HB, Gottschalk RWH, Koppen G, Schoeters G, Baeyens WF, van Larebeke NA, van Delft JHM, Kleinjans JCS (2008) Multiplex genotyping as a biomarker for susceptibility to carcinogenic exposure in the FLEHS biomonitoring study. Cancer Epidemiol Biomarkers Prev 17(8); 1902–1911.

Lee JT, Shy CM (1999) Respiratory function as measured by peak expiratory flow rate and PM_{10}: six communities study. J Exp Anal Environ Epidem 9; 4: 293–9.

Miller AB (1996) Review of extant community-based epidemiologic studies on health effects of hazardous wastes. Toxicol Ind Hlth 12; 2: 225–33.

MOEF (2010) (accessed on7-10-2010) Modified draft E waste (Management and handling) rules-2010 (updated4-10-2010), Ministry of Environment and Forests, Govt. of India. Available from http://moef.nic.in.

Nnorom IC, Osibanjo O (2008) Overview of electronic waste (E-waste) management practices and legislations, and their poor applications in the developing countries. Resources Conservation and Recycling 52: 843–858.

Pandve HT (2007) E-**w**aste management in India: An emerging environmental and health issue. Indian J Occup Environ Med 11: 3:116.

Pinto VN (2008) E-waste hazard: The impending challenge. Ind J Occup Environ Med 12(2): 65.

Rothman KJ, Greenland S (1998) Modern Epidemiology. Lippincott Williams & Wilkins, 3rd edition.

Sahu KK, Agarwal A (2008).Environmental friendly recycling of electronic waste. A challenge before developing countries. J Metallurgy Materials Sci 50(2):107–118

Saffron L, Giusti L, Pheby D (2003) The human health impact of waste management practices - A review of the literature and an evaluation of the evidence. Management of Environmental Quality: An International Journal 14(2):191–213.

Sever LE (1997) Environmental contamination and health effects: what is the evidence? Toxicol Ind Hlth 13:2–3: 145–61.

Shy CM, Degnan D, Fox DL, Mukerjee S, Hazucha MJ, Boehlecke BA, Rothenbacher D, Briggs PM, Devlin RB, Wallace DD (1995) Do waste incinerators induce adverse respiratory effects? An air quality and epidemiological study of six communities. Environ Hlth Persp 103; 7–8: 714–24.

Shy CM, Degnan D, Fox DL, Mukerjee S, Hazucha MJ, Boehlecke BA, Rothenbacher D, SVTC (Silicon Valley Toxic Corporation) (2009) Accessed on 13-11-2009. Poison PCs/Toxic TVs Executive Summary. http://www.svtc.org/cleancc/pubs/pctvexecsum.htm.

Tata Strategic Management Group (2010) (accessed on 30-1-2010) E waste: opportunity of burden. Available from www.tsmg.com/download/article/E-wastever%20final.pdf

TERI (2006) E-waste and its management in India. TERI Infor Dig Energy Environ 5(2): 160–164.

Toxics Link (2010) Accessed on 3-1-2010. E -aste is a health hazard. Available from http://www.toxicslink.org/tnews-view.php?id=9.

Urban Hazards: Mumbai choking on E-waste (2010) (accessed on 26-3-2010) Available from http://www.timesofindia.indiatimes.com/NEWS/India/Urban_hazard_Mumbai choking on E-waste/RssArticle/ articleshow/ 1671262.

Vineis P, Alavanja M, Garte S (2004) Dose-response relationship in tobacco-related cancers of bladder and lung: a biochemical interpretation. Int J Cancer 108: 2–7.

Vineis P, Kriebel D (2006) Causal models in epidemiology: past inheritance and genetic future. Environ Health 5:21.WHO (1998) Health Effects from Landfills: Impact of the Latest Research – Report on a WHO Meeting, WHO Regional Office for Europe, Copenhagen.

Vrijheid M (2000) Health effects of residence near hazardous waste landfill sites: a review of epidemiologic literature. Environ Hlth Persp 108; 1: 101–12.

Wen S, Yang FX, Gong Y, Zhang XL, Hui Y, Li JG, Liu AL, Wu YN, Lu WQ , Xu Y (2008) Elevated levels of urinary 8-hydroxy-2′-deoxyguanosine in male electrical and electronic equipment dismantling workers exposed to high concentrations of polychlorinated dibenzo-p-dioxins and dibenzofurans, polybrominated diphenyl ethers, and polychlorinated biphenyls. Environ Sci Technol 42 (11): 4202–4207.

WHO (1998) Health effects from landfills: Impact of the latest research – report on a WHO Meeting, WHO Regional Office for Europe, Copenhagen.

Wong MH, Wu SC, Deng WJ, Yu XZ, Luo Q, Leung AOW, Wong CSC, Luksemburg WJ, Wong AS (2007) Export of toxic chemicals: A review of the case of uncontrolled electronic waste recycling. Environ Poll 149:2: 131–140.

Zabrosky J (2010) (accessed on 5-1-2010) Electronics recycling lowers my taxes. Available from http://ezinearticles.com/?Electronics-Recycling-Lowers-My-Taxes?&id=2969118.

Zheng L, Wu K, Li Y, Qi Z, Han D, Zhang B, Gu C, Chen G, Liu J, Chen S, Xu X, Hu O (2008) Blood lead and cadmium levels and relevant factors among children's from an e waste recycling town in China. Environ Res 108: 15–20.

Aerospace Toxicology Overview: Aerial Application and Cabin Air Quality*

Arvind K. Chaturvedi

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1 Introduction

The environment extending above and beyond the surface of the planet Earth is referred to as aerospace (Blashfield and Johnson 1969). This word also symbolizes the joint fields of aeronautics and astronautics. The former is the art and science of flight through the atmosphere (Blashfield and Johnson 1969) and the latter is the art and science of space flight (Blashfield and Johnson 1968a). Another term frequently and interchangeably used with aerospace and aeronautics is aviation, which is defined as the art and science of operating powered aircraft (Chaturvedi 2010a, b; The Encyclopedia Americana 1989).

Toxicology is defined as being "the basic science of poisons" and deals with the adverse effects of substances on living organisms; any chemical substance is recognized as potentially being poisonous, although the induction of toxicity is exposure-, amount-, and frequency-dependent (Eaton and Klaassen 1996; Gallo 1996; Loomis 1978). Toxicology is a multidisciplinary subject and acquires and integrates knowledge from biology, chemistry, immunology, pathology, physiology, and public health. The field most closely related to toxicology is pharmacology (Loomis 1978). Toxicology can be divided into subareas referred to as economic, environmental, or forensic toxicology, among others. Thus, aerospace toxicology can be considered to be closely related to aerospace medicine. This medical field is newly emerged and is a specialty area of general medicine (Blashfield and Johnson 1968b). Aerospace toxicology is concerned with the health and medical issues of man in aviation and during space flights (National Library of Medicine 2008). Aerospace medicine can be viewed as the branch of preventive medicine that addresses the special problems of flying, both within and outside the atmosphere (Case University 2008).

To prepare this review, I performed a literature search for the period from 1960 to 2007; the scope of the search covered aerospace toxicology-related subspecialties, agricultural aviation (aerial application) and aircraft cabin air quality. Overviews of other subspecialties – aviation combustion toxicology and postmortem aviation forensic toxicology – have been included in separate scientific articles (Chaturvedi 2010a, b). In the present article, I address the safety of aerially applied chemicals, cabin air quality in aviation, and the harmful effects of fumes and smoke, including the toxicants that may exist in space vehicle cabin air. During the course of the review, I also address chemical exposure monitoring, exposure monitoring methods, aspects of agricultural aviation, in general, and the application of potentially toxic agricultural chemical active ingredients in combinations and with component chemicals (organic solvents and surfactants), in the context of agricultural aviation. Furthermore, in this review I emphasize the potential for the presence of chemical constituents and pyrolysis products of engine oils, hydraulic fluids, and lubricants in aircraft cabin air, and suggest the need for a thorough evaluation of oil additives used in aircraft.

2 Agricultural Aviation

2.1 Application of Chemicals in Agriculture

The use of aerial application is increasing throughout the world to help address increasing food production needs. Many types of agricultural chemicals are applied to crops, crop land, pastures, rangeland, or forests by hand, or by ground or aerial equipment; such agents include insecticides, herbicides, growth modifiers, fertilizers, and others. Many of the chemicals used can be toxic to human beings and may cause serious symptoms and possibly death (Patterson and Rayman 1996). Handling of commercial agricultural chemical preparations (formulations) is involved during aerial application programs, and application incidents and accidents do occur. Some actual examples of such incidents are (1) an experienced aerial applicator pilot, who accidentally spilled parathion on his clothes while pouring the concentrate from a 55-gallon drum four days earlier, and who afterward became irritable and introverted, was not feeling well, and had a headache on the day he crashed his plane, (2) a pilot, who was exposed to drifting parathion and required atropine therapy, flew into a tree during pull-up, (3) an aircraft connector loosened after takeoff and resulted in the spraying of a mixture containing parathion in the pilot's face, saturating his body, and causing him to lose control of the plane, which crashed, and (4) a pilot was splashed with a defoliant during a flight, which caused a crash, almost resulting in the pilot's death (Mohler and Harper 1966). Such accidental exposures and the development of aerial dust allergies in pilots were topics of a group discussion on protecting agriculture pilots 45 years ago (Mohler and Harper 1966); therefore, this problem is a long existing one. In general, agricultural chemicals are toxic (Cullen and Hill 2006; Ecobichon 1996), and if occupational safety and precautionary measures are not properly taken, exposures of applicator personnel (i.e., aircraft loaders, mixers, and agricultural aircraft accident investigators) to such chemicals could lead to acute or chronic poisonings. In addition, poisonings of working agricultural pilots may contribute to aviation accidents. Such poisoning incidents, when they occur, may result from exposures to a single or multiple chemicals (or chemical mixtures).

2.2 Pesticidal Toxicology and Agricultural Aviation

The toxicology of organophosphorus and organochlorine insecticides has been well covered in the literature (Cullen and Hill 2006; Lauwerys 1996). Although no longer used except for malarial programs in Africa, dichlorodiphenyltrichloroethane (DDT) is the most studied organochlorine insecticide, and has also served

as a prototype for the types of toxicological properties that may exist for many other organochlorine insecticides (Cullen and Hill 2006; Lauwerys 1996). Organophosphorus and organochlorine chemicals adversely affect the functions of the central nervous system, though they do so by different mechanisms. Behavioral difficulties have been experienced by aerial applicators following their exposures to certain pesticides (Dille and Smith 1963, 1964; Lewis et al. 1972; Smith et al. 1968; Wood et al. 1971). Symptoms of such exposures have included anxiety, uneasiness, depression with weeping, dizziness, emotional liability, frequent and severe disagreement with family members and coworkers, and being unable to perform familiar tasks. These effects were reported during medical evaluations of two agricultural pilots actively engaged in the aerial application of organophosphorus (methyl parathion) or organochlorine insecticides (DDT, toxaphene, endrin, and dieldrin) (Dille and Smith 1963, 1964).

Toxicological evaluation of postmortem samples from pilots killed while engaged in aerial application revealed that blood cholinesterase levels in 44 of 104 pilots (Lacefield et al. 1975) and 77 of 130 pilots (Lacefield et al. 1978) were below the normal range. This suggests a problem of acute and/or chronic toxicity from exposure to the organophosphorus pesticides these pilots were applying. Reduced plasma cholinesterase levels were found in two agriculture pilots who were involved in nonfatal aviation accidents (Dille and Morris 1966, 1967). The types of accidents and poisonings that have occurred as a result of aerial applications have been documented (Dille and Mohler 1968; Dille and Morris 1966, 1967). Aerial application-related precautions, signs, and symptoms of pesticide poisonings, and their treatments have also been summarized in the literature to help protect agricultural pilots (Dille and Mohler 1968; Mohler and Harper 1966). Among the precautions suggested is a key one that points out the need for better educational efforts designed to reduce accidents in this sector of agricultural activity.

Aerial spraying programs are also used to help manage insect infestations of large tracts of forest. In this area, extensive studies have been conducted on the toxicity of forest insecticides (fenitrothion and aminocarb), the technology of aerial spraying, the development of less hazardous formulations, and the quantitation of off-target drift of aerosolized insecticides (Ecobichon 1990). These studies resulted in improved pesticide application techniques, and they have fostered the establishment of regulations to implement buffer zones around human habitation for certain types of aircraft that apply different formulations of forest insecticides.

2.3 Exposure to Multiagricultural Chemicals and Organic Solvents/Surfactants

If proper safety and precautionary measures are not observed, there is a clear potential for aerial applicators, associated personnel, and aircraft accident investigators to be exposed to multiple agricultural chemicals and the solvents/surfactants in their commercial spray preparations. Such exposure may result in poisonings and could

be produced by any one ingredient, or interactive effects among several active ingredients, or other components of a formulation to which exposure occurs.

During the course of 7- and 14-day treatments, the toxic effects in mice of mixtures of parathion (5 mg/kg), toxaphene (50 mg/kg), and/or dichlorophenoxyacetic acid (2,4-D; 50 mg/kg) were observed to emulate the effects exhibited by the individual components (Kuntz et al. 1990). Metabolic aspects of these three chemicals suggest that the toxicity of the parathion plus toxaphene mixture would be lower than that of parathion, as toxaphene has the ability to increase aliesterases and the biotransformation of parathion to paraoxon, thereby providing a pool of noncritical enzymes for the binding of paraoxon (Chaturvedi et al. 1991). Because of these properties of toxaphene, it is anticipated that the toxicity of a mixture of parathion plus toxaphene plus 2,4-D would also be lower than that of parathion alone (Chaturvedi et al. 1991). Chronic studies in mice on the mixtures of three commonly used herbicides – alachlor, atrazine, and/or picloram – suggest that the mixtures may cause hepatotoxicity and stimulate the liver xenobiotic-metabolizing enzymes (Chaturvedi 1993a). A chronic toxicological evaluation of mixtures of ten widely used pesticides – alachlor, aldrin, atrazine, 2,4-D, DDT, dieldrin, endosulfan, lindane, parathion, and toxaphene – in mice revealed that these mixtures induce the xenobiotic-metabolizing enzymes in liver. Therefore, exposures to such pesticidal mixtures may cause deleterious effects in other species, including humans, by enhancing the metabolism of xenobiotics (Chaturvedi 1993b).

In multichemical exposures, interactive effects among chemicals to which exposure occurs may play a contributory role toward the associated poisonings. This type of poisoning could be exemplified by citing two actual examples: first, a multichemical death that involved caffeine, nicotine, and malathion (Chaturvedi et al. 1983), and another death attributed to ingestion of malathion insect spray (Chaturvedi et al. 1989). In the later case, in vitro inhibition of cholinesterases and the presence of xylenes and other volatiles, in certain postmortem samples, were demonstrated (Chaturvedi et al. 1989). Therefore, these organic solvents may not only interact with other mixture-chemicals, but may also exhibit their own toxic effects.

Ethylbenzene, a major component of mixed xylenes, is used as solvents in agriculture insecticide sprays and has been found to increase the incidences of renal tubule, alveolar/bronchiolar, and hepatocellular neoplasms, and of testicular and renal tubule adenomas, in rats (US National Toxicology Program 1999). Increased incidences of renal tubule hyperplasia of alveolar epithelial metaplasia, and of severe nephropathy have been reported in rats exposed to ethylbenzene. The herbicide glyphosate, though it does not bioaccumulate, biomagnify, or persist in a biologically available form in the environment, and is nontoxic to animals, is formulated with surfactants (Solomon and Thompson 2003). Such formulations increase the efficacy of the herbicide but, in some cases, are more toxic to aquatic organisms than is the parent material. Some risks were observed for measured concentrations of glyphosate in surface waters that resulted from aerial application to forests of a formulation equivalent to Roundup® in Canada.

2.4 Aerial Application Safety

Aviation authorities have long been concerned about the toxic effects of agricultural chemicals on agriculture pilots. In the former Soviet Union, aerial applicators were required to maintain records of the chemicals used for crop spraying and the duration of spraying (Cullen and Hill 2006). In the USA, toxicological problems associated with aerial applications were recognized in the early 1960s, and a considerable number of applied studies were conducted at the US Department of Transportation Federal Aviation Administration's (FAA's) Civil Aerospace Medical Institute in Oklahoma City, OK, to enhance the safety of agricultural pilots and their support personnel. The cogent studies conducted at the FAA's Civil Aerospace Medical Institute are summarized in Table 1.

2.5 Agricultural Chemical Exposure Monitoring

The health risk of aerial spraying is well known for pilots and ground maintenance workers. Therefore, such agricultural workers in the aerial spraying industry must be placed on occupational surveillance programs designed to detect the earliest toxic exposures to these chemicals. Since organophosphorus compounds and carbamates inhibit acetylcholinesterase and other cholinesterases, activities of these enzymes in red blood cells, plasma, or whole blood (30–50% inhibition) are routinely measured for monitoring exposures to these insecticides (Cullen and Hill 2006; Gossel and Bricker 1994a; Lauwerys 1996). In addition, residues of pesticides or their metabolites in body fluids may be measured directly. Examples of tentative maximum permissible concentrations for parent pesticides and/or their metabolites are (1) 0.5 mg of *p*-nitrophenol per g of creatinine in urine for parathion and 10 mg of naphthol per g of creatinine in urine for carbaryl, (2) 15 μg of dieldrin per 100 mL of blood, 2 μg of lindane per 100 mL of blood, and 5 μg of endrin per 100 mL of blood, (3) 30 μg of hexachlorobenzene per 100 mL of blood and/or presence of 2,4,5-trichlorophenol in urine. (4) 0.05 mg of pentachlorophenol per 100 mL of plasma and/or 1 mg of pentachlorophenol per g of creatinine in urine, and (5) detection of 2,4-D and 2,3,5-trichlorophenoxyacetic acid in urine (Lauwerys 1996).

3 Cabin Air Contamination

3.1 Aviation Cabin Air Quality

The quality of air in aircraft cabins has been a topic of debate and discussion since at least the 1970s. Aerospace air pollution issues – that is, cabin air quality of aircraft and space vehicles – have been succinctly addressed in an article by Patterson and Rayman (1996). These issues are viewed in the context of the fact that crews

Table 1 A summary of aerial application-related studies conducted at the Civil Aerospace Medical Institute

Agent(s) or topic	Summary	Reference
Lindane and dieldrin	Alterations in several biochemical values of rat tissues by chronic exposures to lindane and changes in the uptake of L-methionine by chick heart and liver cells by chronic exposure to dieldrin	Daugherty et al. (1962)
Analysis of hazards in the aerial application	Discussion on the nature of the chemicals, the symptoms of toxicity, recommended treatment, and suggestions for safe handling of toxic pest-control chemicals	Smith (1962)
Cardiovascular effects of endrin	Causation of bradycardia, hypertension, salivation, hyperexcitability, tonic–clonic convulsions, increased body temperature, leukocytosis, and decreased blood pH by endrin, appeared to be caused by direct action on the central nervous system	Emerson et al. (1963)
Dieldrin, lindane, heptachlor, isodrin, and endrin	Reduction in the esterification of inorganic phosphate by 50%, without affecting lactic acid production in chickens and rats exposed to dieldrin, but no such reduction in esterification by other chlorinated pesticides lindane, heptachlor, isodrin, and endrin	Daugherty et al. (1963)
Cases involving aerial application of organophosphorus insecticides	Signs/symptoms of anxiety, uneasiness, depression, weeping, dizziness, emotional liability, disagreement with family/coworkers, and unable to perform familiar tasks	Dille and Smith (1963)
Chronic and acute effects of endrin on renal function	Systemic hypertension and increased renal vascular resistance in dogs by acute exposure to endrin, attributed to a sympathoadrenal action Development of progressive systemic hypotension with variable changes in renal function and terminal renal vasodilatation in dogs chronically exposed to endrin Note: These findings were related to hemodynamic alterations in the peripheral vasculature. No evidence of renal failure was observed due to chronic insecticide poisoning.	Reins et al. (1963)
Effects of endrin and carbon tetrachloride	Reversible increase in hepatic fat contents of rats treated with endrin and carbon tetrachloride	Clark (1966a)
Pathological effects of endrin and dieldrin	More severe effects of dieldrin on the cold-adapted rats than on the room-temperature rats	Clark (1966b)
Effects of endrin on renal function and hemodynamics, peripheral vascular system, venous return and catecholamine release, and the cardiovascular system	Exploration of the effects of endrin in dogs on renal function and hemodynamics, peripheral vascular system, venous return and catecholamine release, and the cardiovascular system; and elucidation of the mechanisms of endrin-induced hemoconcentration	Hinshaw et al. (1966)

(continued)

Table 1 (continued)

Agent(s) or topic	Summary	Reference
Exposure of parathion and development of allergy toward aerial dusts	Group discussion on protecting agriculture pilots	Mohler and Harper (1966)
Human factors in general aviation accidents	Discussion on the role of medical conditions and pesticides in aviation accidents	Dille and Morris (1966, 1967)
Cholinesterase measurement	Development of an automated method for measuring cholinesterase activity in blood and tissues of animals poisoned with organophosphates and carbamates	Fowler and McKenzie (1967)
Drug and toxic hazards in general aviation	Summary of aerial application related precautions, signs and symptoms of pesticide poisoning, and their treatments	Dille and Mohler (1968)
Effects of disulfoton	Increase in performance in rats given disulfoton at 10, 25, and 50 ppm in diet and water ad libitum and inhibition of brain acetylcholinesterase by more than 75% of normal in the most severely exposed group	Pearson et al. (1969)
Cholinesterase methods	Comparison of three cholinesterase methods Importance of cholinesterase tests in applicators prior to the aerial application season	Crane et al. (1970a)
Human blood cholinesterase	Decrease of only 10% of the original cholinesterase activity upon storage of red cell hemolysates for less than 12 h at room temperature, up to 3 days at 4°C, and up to 6 weeks at −20°C Note: Whole-blood hemolysates and plasma may be stored for 6 days at room temperature and 6 weeks, if refrigerated or frozen.	Crane et al. (1970b)
Effects of endrin on brain	Brain bioelectric phenomena caused by endrin at doses well below those causing seizures or other gross behavioral changes Seizures in squirrel monkeys by chronic administration of this pesticide Reoccurrence of seizures some months after the termination of endrin administration, under stressful conditions, suggesting that this phenomenon may have been caused by a stress-induced release of endrin from adipose tissue storage sites Discussion on some implications of these findings for aerial applicators	Revzin (1970)
Disulfoton	Adverse effects on the reproduction system in rats by disulfoton Note: The number of pregnancies was decreased in the animals receiving this pesticide. Such decrease could have been attributed to such factors as alteration in the estrus cycles, the receptivity of the female animals, and decrease in sperm concentration or viability.	Ryan et al. (1970)

(continued)

Table 1 (continued)

Agent(s) or topic	Summary	Reference
Serum or plasma cholinesterase methods	Evaluation of four serum or plasma cholinesterase methods and relationships for interconversion among their respective units Emphasis on the importance of the cholinesterase test in applicators prior to the aerial application season	Crane et al. (1972)
Effects on performance of pigeons and monkeys of phosdrin (mevinphos), a cholinesterase inhibitor	A dose related decrease in response rate with the animals and decrements in behavior at doses below which external symptoms of phosdrin poisoning occurred	Lewis et al. (1972)
Mevinphos (phosdrin)	Inhibition of the amplitude of hippocampal-evoked potentials in squirrel monkeys by mevinphos (phosdrin) in the dose range of 0.05–0.2 mg/kg, with no peripheral signs of poisoning such as tremor and salivation Emphasis that mevinphos produces changes in brain function in the absence of the peripheral symptomatology usually taken as indicators of poisoning by aerial applicator personnel Conclusion that exposure to mevinphos may be unexpectedly hazardous since the aerial applicators may be unaware that they have been poisoned	Revzin (1973a)
Mevinphos poisoning with atropine	Based upon squirrel monkey experiments, potentially hazardous dysfunctions of visual perception in aerial applicator personnel being treated for mevinphos poisoning with atropine	Revzin (1973b)
Toxicological findings in fatal civil aviation accidents (1968–1974)	Blood cholinesterase activity below the lower limit of the normal range in 44 of the 104 aerial applicator pilots	Lacefield et al. (1975)
Chlordimeform	Little or no extra risk in aerial applicators (or others) should they be taking *p*-chlorophenylalanine, DL-α-methyl-*p*-tyrosine, phentolamine, methysergide, and phenylephrine during potential exposure to chlordimeform	Smith et al. (1977)
Toxicological evaluation of postmortem samples from 174 pilots killed in aerial application accidents	Incidence of alcohol in specimens similar for agriculture pilots and other general aviation pilots, but the alcohol blood levels tended to be lower in the former category of pilots Evidence of the use of drugs or medications less in agriculture pilots than in other general aviation pilots Cholinesterase levels below normal in the agriculture pilots, suggesting a continuing problem of acute and/or chronic toxicity from pesticides applied by agricultural aircraft	Lacefield et al. (1978)

must work, sleep, and often live in the cabin environments of aircraft and space vehicles. Throughout the world, the possible adverse effects of cabin atmosphere content on the health of air crews and travelers have been evaluated (Brown et al. 2001; Brundrett 2001; Fulton 1985; Harding 1994; Rayman 2001, 2002; Rayman, RB 2001; Vieillefond et al. 1977; Wyss et al. 2001). Congressional bills that relate to aircraft cabin air quality, and a report on the same topic by the US National Academy of Sciences, have addressed the topic of aerospace medicine (Rayman 2001, 2002; Rayman, RB 2002). Before 30 years ago, the quality of cabin air was apparently not an issue in commercial aviation, and the reporting of disease resulting from airborne vectors or toxic fumes was uncommon (Abeyratne 2002).

Modern jetliners may pose a greater threat of disease because their ventilator systems are designed for optimum efficiency, and may lapse in the recycling of clean air, and/or in the effective blocking of engine exhaust fumes that may enter cabin areas. Aerotoxic fumes are most common in the cockpit, and therefore, crew members are the most susceptible to the aerotoxic syndrome (Abeyratne 2002). In a comprehensive review of 21 studies, in which authors examined the effect of the airliner cabin environment and other factors on the health and comfort of flight attendants, Nagda and Koontz (2003) found that various complaints and symptoms reported by the attendants appeared to be associated with their job duties and with the cabin environment. The "dryness" symptoms were attributable to low humidity, and the "fatigue" symptoms to the disruption of circadian rhythm. Certain flight attendant complaints were consistent with possible exposure to air pollutants, but that relationship has not been established because such complaints also were consistent with other causes. Despite health issues associated with air travel, there are enormous benefits of this mode of travel to travelers, to commerce, to international affairs, and to health (DeHart 2003).

Stresses (e.g., airport tumult, barometric pressure changes, immobility, jet lag (Sanders et al. 1999), noise, vibration, and radiation) imposed on travelers by commercial flights, and the capability of US air carriers to deal with in-flight illness and medical care have been addressed in an earlier review article (Rayman 1997). The "cabin air quality" topic has been controversial and of concern to the Aerospace Medical Association (AsMA). As a result, the AsMA has reviewed the scientifically accepted facts associated with the different elements (e.g., pressurization, ventilation, contamination, humidity, and temperature) of aircraft cabin atmospheres (Thibeault 1997). The AsMA recommended that regulators, airlines, and scientific associations work together on the issue of cabin air quality, since technical data alone is inadequate to solve the problem.

Aircraft cabin carbon dioxide (CO_2) concentrations, calculated from the published ventilation ratings, were found to be intermediate to those obtained by actual measurement. These findings were used to arrive at recommendations for aircraft builders and operators to help improve aircraft cabin air quality at minimum cost (Hocking 1998). Several factors were considered that pertained to cabin air quality before proposals were made. These factors included the trends, effects, and societal costs of cabin air quality on passengers and crew. Improvement was successfully made in cabin air quality that has resulted in a net, multistakeholder savings and improved passenger comfort (Hocking 2000). Aviation-industry and

passenger perspectives on cabin air quality have been evaluated by Hocking (2002). Accordingly, recommendations and suggestions were made for aircraft builders, operators, and passengers. These recommendations were designed to help improve aircraft cabin air quality, to improve the partial pressure of oxygen that is available to passengers at minimal cost, and to enhance passenger comfort and decrease risk of illness. Rayman, RB (2002) made recommendations on how the cabin air quality issue may be resolved, whereas Thibeault (2002) argued in a review that airliner cabin air quality was adequate and did not compromise the health of aircrews, though this author acknowledged the need for further studies.

3.2 Harmful Effects of Aircraft Cabin Air

Air crew fatigue for those performing frequent and long flights has been linked to effects from aircraft-related noise, temperature, cabin pressure, ventilation, atmosphere quality, humidity, and jet lag, among other flight characteristics (Fulton 1985; Vieillefond et al. 1977). Fulton (1985) addressed the effects of ventilation adequacy, cigarette fires, and pilot health issues in aircraft cabins. Harding (1994) acknowledged that the amounts of fresh air in aircraft cabins may be marginal, but there was nonetheless sufficient oxygen for human consumption. The concentration of microorganisms in airline cabin air was found to be much lower than concentrations in ordinary city locations (Wick and Irvine 1995). Hence, it was concluded that the small number of microorganisms found in US airliner cabin environments does not contribute to the risk of disease transmission among passengers.

In a 1997 study of Airbus aircraft (Dechow et al. 1997), the number of particles in cabin air was compared with those found in fresh air and recirculated air. In addition, levels of microbiological contamination and volatile organic compounds were investigated in cabin air. Results indicated that particles were mainly emitted by passengers, especially smokers, and particle counts in recirculated air were lower or equal to those occurring in fresh air. By contrast, bacterial counts in the aircraft cabin exceeded those in fresh air. The detected microbes were mainly nonpathogenic and the concentrations of volatile organic compounds were well below threshold values. Modern high-efficiency particulate air (HEPA)-filters are used in aircraft and minimize the accumulation of bacteria and viruses in recirculated cabin air. Such HEPA filtration in aircraft significantly reduces the overall risk of acquiring infectious diseases, compared with other means of transportation (Bergau 1999).

The issue of the flying fitness of patients, who have infections, has also been addressed (Haditsch 2002). Aircraft that carry both cargo and passengers have been implicated in disease transmission, since they may transport humans, along with mosquitoes or other insect disease vectors, and animals (DeHart 2003). Events of tuberculosis and influenza transmission to other travelers have been reported, and the vectors of yellow fever, malaria, and dengue have been identified on aircraft. However, studies of the ventilation systems and patient outcomes suggest that the spread of pathogens rarely occurs during flights (Leder and Newman 2005).

A review of the concentrations of organic compounds in cabin air has indicated that contaminant levels are similar to those that exist in residential and office buildings (Nagda and Rector 2003). However, there were two exceptions. First, levels of ethanol and acetone – indicators of bioeffluents and chemicals from consumer products – were higher in aircraft air than in home or office environments, or in other transportation modes; second, levels of certain chlorinated hydrocarbons and fuel-related contaminants were higher in residential/office buildings than in aircraft air. The levels of the *m*- and/or *p*-xylenes tend to be lower in aircraft. Although cabin air is filtered through adsorbents, prior to recirculation, to remove volatile organic compounds and odor, such devices are not installed in all aircraft and may not be capable of removing all pollutants. Therefore, the photocatalytic air filtering approach was developed and this approach seems to be a promising method to resolve odor problems in aircraft (Ginestet et al. 2005). This photocatalytic unit consists of four UV lamps sandwiched between two interchangeable titanium dioxide-coated panels and is designed to oxidize volatile organic compounds. The overall efficiency of the catalytic unit was dependent upon the chemical characteristics of the compounds that were used to challenge the unit. The compounds used were toluene, ethanol, and acetone. The tested unit did not fully remove toluene, since the unit relies on oxidation to remove substances, and toluene is the most difficult compound to be oxidized. Moreover, although the tested prototype unit is able to partially oxidize volatile organic compounds, partial oxidation of some toxic intermediate chemical reaction products may result in the production of intermediates such as formaldehyde and acetaldehyde.

High concentrations of ozone in cabin air can lead to upper respiratory problems, and inhaling the high levels of CO_2 that may occur in cabin air may produce hyperventilation (Bergau 1999). Breathing cabin air may also cause the mucous membranes of the respiratory tract to dry out because of the extremely low humidity of cabin air. In a 2000 study by Backman and Haghighat (2000), air quality in 15 different aircraft was measured at different times and altitudes. High CO_2 concentrations and low humidity levels were found in the Airbus 320 aircraft. The highest humidity level was found in the DC-9 aircraft and the lowest CO_2 concentration was analyzed in the Boeing 767 aircraft. The authors concluded that poor air quality may cause intolerance to contact lenses, and dry eyes, and may be a health hazard to both passengers and crew members. In the US Air Force C-5 aircraft cabin air, carbon monoxide (CO) and CO_2 concentrations were found to be well below health effect thresholds, whereas the lowest level of relative humidity found was 3%, and ozone existed at relatively low concentrations (Hetrick et al. 2000). The influence of ozone on self-evaluation of symptoms in a simulated aircraft cabin indicated that air quality, as measured by the presence or absence of 12 symptoms (e.g., eye and nasal irritation, lip and skin dryness, headache, dizziness, mental tension, and claustrophobia), was established to be significantly worse ($p < 0.05$) for the 60–80 ppb ozone atmosphere ("ozone" condition), compared to the <2 ppb ozone atmosphere ("no ozone" condition) (Strom-Tejsen et al. 2008).

During intercontinental flights, CO_2 levels were below 1,000 ppm in 97% of the cases and humidity was very low (mean 5%) (Lindgren et al. 2000). Low humidity

in aircraft cabins is further demonstrated to be a factor for the mucosal irritation experienced by travelers and flight attendants (Lindgren and Norback 2002; Nagda and Hodgson 2001; Uva Ade 2002), and tobacco-smoking onboard may contribute to significant pollution from respirable dust (Lindgren and Norback 2002; Lindgren et al. 2000; Wieslander et al. 2000). Lindgren et al. (2007) investigated the influence of air humidification during intercontinental flights on the perception of cabin air quality among airline crew. These authors concluded that relative humidity can be slightly increased by using a ceramic evaporation humidifier, without showing any measurable increase of microorganisms (Lindgren et al. 2007). Their evaluation of the optimum balance between fresh air supply and humidity, involving 7-h exposures in a simulated aircraft cabin, indicated that increasing the relative humidity to 28% by reducing outside flow to 1.4 L/s per person did not reduce the intensity of the symptoms that are typical of the aircraft cabin environment. However, this adjustment intensified complaints of headache, dizziness, and claustrophobia that resulted from the increased level of contaminants (Strom-Tejsen et al. 2007).

The contribution of secondhand tobacco smoke to aircraft cabin air pollution was assessed for flight attendants, and compared to results from the general population; results indicated that ventilation systems massively failed to control secondhand smoke air pollution in cabins (Repace 2004). However, smoking is now prohibited by most airlines, and the pollution caused by smoking is no longer a relevant issue. The authors of another study emphasized that the relative air humidity of cabin air was very low on intercontinental flights, and particle levels were high on flights with passive smoking (Lindgren and Norback 2005). These findings suggested the need for improving cabin air quality by better controlling cabin temperature, air humidification, and air filtration (HEPA filters), and having a sufficient air exchange rate on all aircraft types.

3.3 Possible Toxicants in Space Vehicle Cabin Air

Astronauts work, sleep, and live in space vehicles (Patterson and Rayman 1996), and there is a strong potential for a slow and insidious buildup of toxic substances – such as refrigerants, CO, hydrogen cyanide (HCN), CO_2, ammonia, and other organic compounds – in the space-vehicle cabin atmosphere. Also, high concentrations of toxic substances may be rapidly released from onboard fires. The deaths of the three Apollo 1 crew members in the 1967 fire accident resulted from their exposure to toxic combustion products (US National Aeronautics and Space Administration 1967). Moreover, the involvement of fire has been acknowledged in the 23 February 1997 accident on the Mir aerospace station (Welch and Navias 1997), wherein the fire burned for approximately 90 s and the crew was exposed to heavy smoke for 5–7 min.

In addition to the combustion gases (e.g., CO and HCN) originating from fire (Chaturvedi 1995; Chaturvedi and Sanders 1995, 1996; Sanders and Chaturvedi 1994), sources of toxic substances in cabin air can result from off-gassing of space

vehicle material, crew metabolism (CO_2 in particular), payload chemicals, and thermal degradation of materials present in the aircraft (Patterson and Rayman 1996). Therefore, the protection of the astronauts' health and preventing their performance decrements are crucial. A major need in the space cabin is to establish maximum allowable concentrations of potentially toxic substances. Such an effort should be based on the fact that astronauts live in the closed environment of their space vehicles 24 h a day, for weeks or even months, in comparison to the standard 8-h shift worked by most terrestrial workers. Exposure to microbes in the space cabin is also of concern because crew members release many bacteria into the environment, and exudation of aerosols in a microgravity environment results in droplets being suspended in the atmosphere. Both factors render exposures more likely. How microgravity affects the immune system of humans has not been well established. Therefore, monitoring for microorganisms and toxic substances in the space vehicle cabin atmosphere is essential. Surveys have shown that the methods and means of qualitative and quantitative air monitoring on the International Space Station are currently sufficient for air control in emergency situations such as local fire and toxic leak; moreover, the Station's air quality is regarded to be suited to the existing standards and crew safety requirements (Pakhomova et al. 2006).

3.4 Fumes/Smoke in Aircraft Cabins: Analysis and Effects

Pyrolytic products of jet engine oils, hydraulic fluids, and/or lubricants may enter aircraft air by leaking in through ventilation systems (Crane et al. 1983; Sanders 2007; van Netten 1999; van Netten and Leung 2001). When this occurs, exposures to them may impose a threat to the operating aircraft and to their occupants (Rayman and McNaughton 1983). Smoke/fumes-related incidents are usually caused by broken engine seals or associated systems that allow smoke and fumes to enter the air compressor section from where they can contaminate the interior of the aircraft. Catalytic converters have been used to clean the air (van Netten and Leung 2000), but when an oil seal fails, such systems can easily become overloaded, allowing smoke to enter the cabin. The potential for exposure to thermal breakdown products, including smoke or other toxic gases, may cause performance impairment of the crew members. Dizziness, nausea, disorientation, blurred vision, tingling in legs and arms, central nervous system dysfunction, and mucous membrane irritation have frequently been reported by flight crews. Such symptoms are consistent with exposures to CO and some pyrolysis products, including volatile organic compounds and the organophosphate constituents of the oils and fluids, but the involvement of these liquids has not been clearly demonstrated (van Netten 1999).

Smoke consists of particulate matter and a variety of invisible combustion gases and vapors that are suspended in the fire environment, resulting from a rapid exothermic chemical chain reaction between a fuel and oxygen (Landrock 1983; Meyer 1977; Smyth et al. 1992; Strahle 1993). The types of combustion gases produced depend upon the nature of the chemical constituents of the material being burned.

For example, polymers, such as polyethylene produces CO and CO_2; Nylon 6/6 produces CO, HCN, and CO_2; polyamide produces CO, HCN, and CO_2; polystyrene produces CO, CO_2, and benzene; chlorinated polyethylene produces CO, CO_2, and hydrogen chloride; and polysulfone produces CO, CO_2, and sulfur dioxide (Fenner 1975; Harper 1975; Sanders et al. 1991, 1992). Of these, CO and HCN are two primary toxic gases present in smoke (Chaturvedi 1995; Chaturvedi and Sanders 1995, 1996; Sanders and Chaturvedi 1994).

Carbonaceous compounds produce CO and CO_2 upon burning, and nitrogenous compounds also produce HCN (Chaturvedi 1995; Sanders et al. 1991, 1992; Chaturvedi and Sanders 1995, 1996). Because the aircraft structure is composed of a variety of carbon- and nitrogen-containing polymeric materials, there is strong potential for the generated smoke to be rich in CO and HCN. In the absence of fire, the presence of CO in the interior of the aircraft would suggest a malfunctioning of the heating/exhaust systems. Since aviation fuel is primarily a mixture of non-nitrogen-containing hydrocarbons, aircraft engine exhaust would contain a negligible amount of HCN (Chaturvedi et al. 2001).

Exposure of aircraft occupants to CO and HCN can be monitored by analyzing for these gases in the blood as carboxyhemoglobin (COHb) or the cyanide ion (CN^-). Analytical methods for measuring COHb and CN^- are mentioned in an overview (Chaturvedi 2009, 2010a) and in an international standard (ISO International Standard 2008). Those analytical methods are summarized herein.
For COHb:

1. Whole-blood oximetry by simultaneous differential visible spectrometry at various characteristic wavelengths (AVOXimeter 2001; CO-Oximeter 1978; Freireich et al. 1975).
2. Reduction of palladium chloride to palladium by releasing CO from COHb in blood by sulfuric acid and measuring absorbance at 278 nm of the remaining unreacted palladium chloride solution (Williams 1970, 1975; Williams et al. 1960).
3. Visible spectrophotometry by hemolyzing red blood cells by ammonium hydroxide, treating the hemolysate with sodium dithionite to reduce methemoglobin (MetHb) and oxyhemoglobin (OxyHb) to deoxyhemoglobin (HHb), and measuring absorbance at 540 nm, a wavelength of maximum absorbance for COHb, and at 579 nm, a wavelength at which the spectra of various species of HHb have the same absorbance (Blanke 1976a; Canfield et al. 1998, 1999; Douglas 1962; Sanderson et al. 1978; Tietz and Fiereck 1973; Winek and Prex 1981). A ratio of absorbance values at 540 nm and 579 nm is used to determine %COHb in the specimen, with the help of a calibration curve.
4. Visible spectrophotometry by saturating Part 1 of three equal parts of blood hemolysate with CO, and of Part 2 with oxygen (Part 3 was not treated with any gas), adding sodium dithionite to all the three parts to reduce MetHb and OxyHb to HHb, and determining ratios of the absorbance values of the solutions at 540 and 579 nm to find out %COHb in the specimen by using a mathematical relationship (Canfield et al. 1998, 1999; Rodkey et al. 1979; Sanderson et al. 1978; Uges 2004; Widdop 2002; Winek and Prex 1981).

5. Visible spectrophotometry by hemolyzing red cells of blood specimens, reducing MetHb and OxyHb to HHb, and calculating ratios of the absorbance values of the specimens at 540 and 579 nm (Canfield et al. 1998, 1999; Sanderson et al. 1978; Winek and Prex 1981).
6. Headspace gas chromatography by (1) converting MetHb and OxyHb to HHb by using sodium dithionite in two separate aliquots of blood samples, (2) saturating one aliquot with CO (the second aliquot is not treated with CO), (3) getting the release of CO from both aliquots by a ferricyanide or phosphoric acid solution, (4) injecting headspace air samples of the CO-saturated and non-CO treated aliquots into a gas chromatograph, equipped with a column and a methanation unit (nickel catalyst and hydrogen unit), (5) detecting methane by flame ionization, and (6) calculating %COHb level in a blood sample by comparing methane peaks of the CO-saturated blood sample and of the non-CO treated (original) blood sample (Cardeal et al. 1993; Griffin 1979).
7. Headspace gas chromatography by dividing the sample into two parts, saturating one part with CO (other used without CO-treatment), treating both parts with sodium dithionite to reduce MetHb and OxyHb to HHb, releasing CO from the both parts by sulfuric acid with saponin, injecting headspace air samples of the CO-saturated and the non-CO treated samples into a micro-gas chromatograph, and comparing gas chromatographic CO peaks of the original (non-CO treated) blood sample and of the CO-saturated blood sample, and calculating %COHb in a blood sample (Endecott et al. 1996; Lewis et al. 2004).

For CN^-:

1. Colorimetry by the reaction of CN^- present in blood with *p*-nitrobenzaldehyde and *o*-dinitrobenzene under an alkaline condition and production of a violet color, suggesting the presence of a potentially toxic CN^- concentration (Dunn and Siek 1990; Guilbault and Kramer 1966; Rieders 1975a).
2. Visible spectrophotometry by the liberation of HCN from blood by acidification and microdiffusion, trapping of HCN in a dilute alkaline solution, conversion of HCN to cyanogen chloride after reacting with chloramine-T, and then reacting cyanogen chloride and pyridine to form *N*-cyanopyridinium chloride, followed by a cleavage reaction to form an anil of glutaconic aldehydes and then coupling with barbituric acid to generate a red-pinkish, highly resonant product indicating the presence of CN^- (Blanke 1976b; Feldstein and Klendshoj 1957; Rieders 1975b).
3. Headspace gas chromatography (nitrogen-phosphorus detection) by equilibration of blood in the presence of an internal standard (acetonitrile) in a vial and injection of the headspace of the vial onto a gas chromatograph to detect HCN and acetonitrile (McAuley and Reive 1983; Zamecnik and Tam 1987).
4. Headspace gas chromatography (electron capture detection) by the liberation of HCN from blood, conversion of HCN to cyanogen chloride by reaction with chloramine-T, and injection of headspace onto a gas chromatograph (Odoul et al. 1994).
5. Spectrophotofluorimetry or high-performance liquid chromatography (fluorescence detection) by transformation of CN^- by acidification from blood to

HCN, reaction of CN^- in HCN with 2,3-naphthalenedialdehyde and taurine, and fluorimetric measurement ($\lambda_{excitation} = 418$ nm; $\lambda_{emission} = 460$ nm) of the reaction product, 1-cyano-2-benzoisoindole (1-cyano[*f*]benzoisoindole; CBI) derivative (Felscher and Wulfmeyer 1998).

6. High-performance liquid chromatography by using isotopic potassium cyanide ($K^{13}C^{15}N$) as an internal standard, microdiffusion of CN^- and $^{13}C^{14}N^-$ from blood as HCN and $H^{13}C^{14}N$, reaction of CN^- and $^{13}C^{14}N^-$ in HCN and $H^{13}C^{14}N$ with 2,3-naphthalenedialdehyde and taurine to produce nonisotopic and isotopic analogs of CBI, and qualitative and quantitative determination of both CBI analogs by high performance liquid chromatography-mass spectrometric detection (Tracqui et al. 2002).

Signs and symptoms for exposures to CO and HCN, in relation to their respective concentrations as %COHb and blood CN^-, are tabulated in previous publications (Chaturvedi 2009, 2010a; Gossel and Bricker 1994b; ISO International Standard 2008).

Fifteen nonfire aviation accidents, involving 17 fatalities (15 pilots and 2 passengers), were reported during 1991–1998 (Chaturvedi et al. 2001). The levels of COHb in these fatalities ranged from 10 to 69%; CN^- was not detected. The selective presence of COHb in the absence of CN^- and fire in these accidents was hypothesized to result from the inhalation of CO present in the interior, because of the faulty exhaust/heating systems. The source of such CO is incomplete oxidation of aviation fuel. The factors that contributed to these 15 accidents were heating/exhaust system malfunctions, pilot error, and/or CO-induced incapacitation. Of these factors, three accidents, accounting for five fatalities (COHb levels of the five: 12, 24, 41, 43, and 69%), were attributed to CO-induced incapacitation or a defective exhaust system. Of the total fatal accidents (2,837) that occurred during the 8-year period, nonfire, CO-related accidents amounted to only 0.53%.

Elevated COHb levels were reported in 13 of the 2,449 pilots killed in general aviation operations between 1973 and 1977, possibly from faulty heaters or exhaust systems (Lacefield et al. 1978). Many accidents reported in 1981 that involved turboprop aircraft potentially resulted from incapacitation of pilots who had inhaled toxic fumes introduced through the cabin pressurization system (Sanders 2007). In response to these accidents, the thermal (300–600°C) decomposition products from aircraft petroleum-based engine and synthetic lubricating oils were evaluated for time-to-incapacitation and time-to-death in rats; the animals were exposed to smoke from these products (Crane et al. 1983). The decomposition of these oils produced CO in sufficient quantities to produce the toxic responses noted.

The bleed air that is diverted from a location just forward of the jet engine combustion chamber has a temperature of approximately 500°C. Thermal breakdown products of jet engine lubrication oils have not been fully characterized at this temperature. Thus, the temperature stability of two commercially available jet oils was investigated by van Netten and Leung (2000), who analyzed for the release of various volatiles and gases by gas chromatography-mass spectrometry. The results show that >100 ppm CO and some CO_2 were generated after exposing the oils to a temperature of 525°C. Nitrogen dioxide and HCN were not detected. The presence of

the neurotoxic tricresyl phosphates (TCPs) was confirmed in the bulk oils and in the volatiles, but trimethyl propane phosphate (TMPP) was not found in these experiments. The absence of TCPs in cabin air possibly resulted from localized condensation in the ventilation ducts and filters, and in the air-conditioning packs. The possibility of the release of pyrolysis products from localized condensates could not be ruled out, particularly when cabin heat demand is high (van Netten and Leung 2001).

The quality of cabin air associated with a contamination of cabin air supply contaminated with the degradation products of oils and fluids was addressed in a UK study (CAA 2004). In this study, two contaminated cabin air supply ducts were examined and analyzed for the presence of chemical constituents and degradation products of engine oils, hydraulic fluids, and lubricants. The inner surface of the ducts was found to be coated with black carbonaceous particulate material, which could be easily dislodged by gentle pressure. Therefore, the material could potentially have become airborne and emitted as solid aerosols in the cabin and flight deck environment. The material was found to contain aluminum, silicon, sulfur, and phosphorus. Gas chromatographic-mass spectrometric analyses of air samples from the contaminated ducts disclosed the presence of short-chain irritants (carboxylic acids, aldehydes, and ketones). Analyses of the solvent extracts of the black duct material further indicated the presence of high molecular weight compounds such as TCPs, TMPP, trimethylolpropane phosphates, and associated esters, suggesting that these compounds may have been tightly bound to the black material. These findings suggest that not all of the chemicals adsorbed onto the material could be desorbed by airflow (for further discussion of this event also see: Chaturvedi 2009, 2010a).

The absence of the solvent extractable chemicals in duct airflow does not mean that those chemicals present in the airflow are the only chemicals responsible for toxicological effects, because other compounds adsorbed onto the duct's material may be released as particulates and may contribute to the toxicity. Since the particles can easily be dislodged, they could easily enter the aircraft interior when the temperature in the cabin is high, and/or when physical disturbances occur during flights (e.g., takeoffs and landings). If the cabin and flight deck occupants inhale those particles, they would be exposed to any chemical present in the duct airflow, including airborne particulates emitted from solid deposits. Such exposure is likely to cause adverse effects, including ocular and upper respiratory irritation, nausea, vomiting, dizziness, and pulmonary toxicity. Because some of the neurotoxicants involved have delayed effects, some toxic symptoms may not appear in exposed individuals for some time.

Although the toxicity of the substances in the black carbonaceous particulate material found in the ducts is described and discussed with sufficient relevant scientific references in the UK study (CAA 2004), the toxicity of this solid carbonaceous material, as a whole entity, is not given in detail. The chemicals comprising the carbonaceous material may not necessarily be individually toxic at the concentrations found, but if they are mixed together at those concentrations, the mixture may be highly toxic (Eaton and Klaassen 1996). Because of the difficulty of dealing with complex chemical mixtures, including pyrolysis products, the best approach to

resolve this toxicological and aviation safety issue would be to minimize risks by preventing oil leaks into bleed air, and monitoring, cleaning, and/or replacing air ducts on a regular schedule. In addition, a more thorough evaluation of the toxic nature of the oil additives used in aircraft engines would be useful (Nicholson et al. 2003).

4 Summary

Aerospace toxicology is a rather recent development and is closely related to aerospace medicine. Aerospace toxicology can be defined as a field of study designed to address the adverse effects of medications, chemicals, and contaminants on humans who fly within or outside the atmosphere in aviation or on space flights. The environment extending above and beyond the surface of the Earth is referred to as aerospace. The term aviation is frequently used interchangeably with aerospace.

The focus of the literature review performed to prepare this paper was on aerospace toxicology-related subject matters, aerial application and aircraft cabin air quality. Among the important topics addressed are the following:

- Aerial applications of agricultural chemicals, pesticidal toxicity, and exposures to aerially applied mixtures of chemicals and their associated formulating solvents/surfactants
- The safety of aerially encountered chemicals and the bioanalytical methods used to monitor exposures to some of them
- The presence of fumes and smoke, as well as other contaminants that may generally be present in aircraft/space vehicle cabin air
- And importantly, the toxic effects of aerially encountered contaminants, with emphasis on the degradation products of oils, fluids, and lubricants used in aircraft, and finally
- Analytical methods used for monitoring human exposure to CO and HCN are addressed in the review, as are the signs and symptoms associated with exposures to these combustion gases

Although many agricultural chemical monitoring studies have been published, few have dealt with the occurrence of such chemicals in aircraft cabin air. However, agricultural chemicals do appear in cabin air; indeed, attempts have been made to establish maximum allowable concentrations for several of the more potentially toxic ones that are found in aircraft cabin air. In this article, I emphasize the need for precautionary measures to be taken to minimize exposures to aerially encountered chemicals, or aircraft cabin air contaminants and point out the need for future research to better address toxicological evaluation of aircraft-engine oil additives.

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References

Abeyratne R (2002) Forensic aspects of the aerotoxic syndrome. Med Law 21:179–199.
AVOXimeter (2001) AVOXimeter 4000 Manual. A-VOX Systems, Inc., San Antonio, TX.
Backman H, Haghighat F (2000) Air quality and ocular discomfort aboard commercial aircraft. Optometry 71:653–656.
Bergau L (1999) [Radiation exposure and air quality aboard commercial airplanes]. Z Arztl Fortbild Qualitatssich 93:491–494.
Blanke RV (1976a) Analysis of drugs and toxic substances. In: Tietz NW (ed) Fundamentals of Clinical Chemistry, 2nd Ed. W.B. Saunders Co., Philadelphia, PA pp 1105–1109.
Blanke RV (1976b) Analysis of drugs and toxic substances. In: Tietz NW (ed) Fundamentals of Clinical Chemistry, 2nd Ed. W.B. Saunders Co., Philadelphia, PA pp 1116–1118.
Blashfield JF, Johnson RJ (1968a) Above and Beyond: The Encyclopedia of Aviation and Space Sciences. New Horizons Publishers, Inc., Chicago, IL p 212.
Blashfield JF, Johnson RJ (1968b) Above and Beyond: The Encyclopedia of Aviation and Space Sciences. New Horizons Publishers, Inc., Chicago, IL p 292.
Blashfield JF, Johnson RJ (1969) Above and Beyond: The Encyclopedia of Aviation and Space Sciences. New Horizons Publishers, Inc., Chicago, IL p 26.
Brown TP, Shuker LK, Rushton L, Warren F, Stevens J (2001) The possible effects on health, comfort and safety of aircraft cabin environments. J R Soc Health 121:177–184.
Brundrett G (2001) Comfort and health in commercial aircraft: a literature review. J R Soc Health 121:29–37.
CAA (2004) Safety Regulation Group: cabin air quality, Report No. CAA Paper 2004/04 (2004 Feb). Civil Aviation Authority (CAA), West Sussex, UK.
Canfield DV, Smith M, Ritter RM, Chaturvedi AK (1999) Preparation of carboxyhemoglobin standards and calculation of spectrophotometric quantitation constants. J Forensic Sci 44:409–412.
Canfield DV, Smith MD, Ritter RM, Chaturvedi AK (1998) Preparation of carboxyhemoglobin standards and calculation of spectrophotometric quantitation constants, Report No. DOT/FAA/AM-98/21 (1998 Aug). U.S. Department of Transportation, Federal Aviation Administration, Office of Aviation Medicine, Washington, DC.
Cardeal ZL, Pradeau D, Hamon M, Abdoulaye I, Pailler FM, Lejeune B (1993) New calibration method for gas chromatographic assay of carbon monoxide in blood. J Anal Toxicol 17:193–195.
Case University (2008) Aerospace medicine. Retrieved 23 April 2008 from http://www.case.edu/med/epidbio/mphp439/Dictionary.htm.
Chaturvedi AK (1993a) Biochemical and toxicological studies on the mixtures of three commonly-used herbicides in mice. Arch Environ Contam Toxicol 24:449–454.
Chaturvedi AK (1993b) Toxicological evaluation of mixtures of ten widely used pesticides. J Appl Toxicol 13:183–188.
Chaturvedi AK (1995) Smoke!, Report No. AM-400-95/1. U.S. Department of Transportation, Federal Aviation Administration, Civil Aerospace Medical Institute, Aerospace Medical Education Division, Oklahoma City, OK.
Chaturvedi AK (2009) Aerospace toxicology: an overview, Report No. DOT/FAA/AM-09/8 (2009 Apr). U.S. Department of Transportation, Federal Aviation Administration, Office of Aerospace Medicine, Washington, DC.
Chaturvedi AK (2010a) Aviation combustion toxicology: an overview. J Anal Toxicol 34:1–16.
Chaturvedi AK (2010b) Postmortem aviation forensic toxicology: an overview. J Anal Toxicol 34:169–176.
Chaturvedi AK, Kuntz DJ, Rao NGS (1991) Metabolic aspects of the toxicology of mixtures of parathion, toxaphene and/or 2,4-D in mice. J Appl Toxicol 11:245–251.
Chaturvedi AK, Rao NGS, McCoy FE (1983) A multi-chemical death involving caffeine, nicotine and malathion. Forensic Sci Int 23:265–275.

Chaturvedi AK, Sanders DC (1995) Aircraft fires, smoke toxicity, and survival: an overview, Report No. DOT/FAA/AM-95/8 (1995 Feb). U.S. Department of Transportation, Federal Aviation Administration, Office of Aviation Medicine, Washington, DC.

Chaturvedi AK, Sanders DC (1996) Aircraft fires, smoke toxicity, and survival. Aviat Space Environ Med 67:275–278.

Chaturvedi AK, Singh G, Rao NGS, Parker TM (1989) Toxicological evaluation of a poisoning attributed to ingestion of malathion insect spray and correlation with in vitro inhibition of cholinesterases. Hum Toxicol 8:11–18.

Chaturvedi AK, Smith DR, Canfield DV (2001) Blood carbon monoxide and hydrogen cyanide concentrations in the fatalities of fire and non-fire associated civil aviation accidents, 1991–1998. Forensic Sci Int 121:183–188.

Clark G (1966a) Problems in aerial application: a comparison of the acute effects of endrin and carbon tetrachloride on the livers of rats and of the residual effects one month after poisoning, Report No. FAA-AM-66-34 (1966 Jul). U.S. Department of Transportation, Federal Aviation Agency, Office of Aviation Medicine, Washington, DC.

Clark G (1966b) Problems in aerial application: a comparison of the effects of dieldrin poisoning in cold-adapted and room-temperature mammals, Report No. FAA-AM-66-5 (1966 Apr). U.S. Department of Transportation, Federal Aviation Agency, Office of Aviation Medicine, Washington, DC.

CO-Oximeter (1978) Operator's manual IL282 CO-Oximeter. Instrumentation Laboratory, Inc., Lexington, MA.

Crane CR, Sanders DC, Abbot JK (1970a) A comparison of three serum cholinesterase methods, Report No. FAA-AM-70-13 (1970 Aug). U.S. Department of Transportation, Federal Aviation Administration, Office of Aviation Medicine, Washington, DC.

Crane CR, Sanders DC, Abbot JK (1970b) Studies on the storage stability of human blood cholinesterases: I, Report No. FAA-AM-70-4 (1970 Jan). U.S. Department of Transportation, Federal Aviation Administration, Office of Aviation Medicine, Washington, DC.

Crane CR, Sanders DC, Abbot JK (1972) A comparison of serum cholinesterase methods: II, Report No. FAA-AM-72-12 (1972 Mar). U.S. Department of Transportation, Federal Aviation Administration, Office of Aviation Medicine, Washington, DC.

Crane CR, Sanders DC, Endecott BR, Abbott JK (1983) Inhalation toxicology: III. evaluation of thermal degradation products from aircraft and automobile engine oils, aircraft hydraulic fluid, and mineral oil, Report No. FAA-AM-83-12 (1983 Apr). U.S. Department of Transportation, Federal Aviation Administration, Office of Aviation Medicine, Washington, DC.

Cullen SA, Hill IR (2006) Aviation pathology and toxicology. In: Rainford DJ, Gradwell DP (eds) Ernsting's Aviation Medicine, 4th Ed. Hodder Arnold, London, UK pp 517–532.

Daugherty JW, Lacey DE, Korty P (1962) Problems in aerial application: I. some biochemical effects of lindane and dieldrin on vertebrates, Report No. FAA-AM-62-10 (1962 May). U.S. Department of Transportation, Federal Aviation Agency, Civil Aeromedical Research Institute, Oklahoma City, OK.

Daugherty JW, Lacey DE, Korty P (1963) Problems in aerial application: II. effects of chlorinated hydrocarbons on substrate-linked phosphorylation, Report No. FAA-AM-63-4 (1963 Mar). U.S. Department of Transportation, Federal Aviation Agency, Civil Aeromedical Research Institute, Oklahoma City, OK.

Dechow M, Sohn H, Steinhanses J (1997) Concentrations of selected contaminants in cabin air of airbus aircrafts. Chemosphere 35:21–31.

DeHart RL (2003) Health issues of air travel. Annu Rev Public Health 24:133–151.

Dille JR, Mohler SR (1968) Drug and toxic hazards in general aviation, Report No. FAA-AM-68-16 (1968 Sep). U.S. Department of Transportation, Federal Aviation Administration, Office of Aviation Medicine, Washington, DC.

Dille JR, Morris EW (1966) Human factors in general aviation accidents, Report No. FAA-AM-66-27 (1966 Jul). U.S. Department of Transportation, Federal Aviation Agency, Office of Aviation Medicine, Washington, DC.

Dille JR, Morris EW (1967) Human factors in general aviation accidents. Aerosp Med 38:1063–1066.
Dille JR, Smith PW (1963) Central nervous system effects of chronic exposure to organophosphate insecticides, Report No. FAA-AM-63-24 (1963 Oct). U.S. Department of Transportation, Federal Aviation Agency, Civil Aeromedical Research Institute, Oklahoma City, OK.
Dille JR, Smith PW (1964) Central nervous system effects of chronic exposure to organophosphate insecticides. Aerosp Med 35:474–478.
Douglas TA (1962) The determination of carbon monoxide in blood. Ann Occup Hyg 5:211–216.
Dunn WA, Siek TJ (1990) A rapid, sensitive, and specific screening technique for the determination of cyanide. J Anal Toxicol 14:256.
Eaton DL, Klaassen CD (1996) Principles of toxicology. In: Klaassen CD, Amdur MO, Doull J (eds) Casarett & Doull's Toxicology: The Basic Science of Poisons, 5th Ed. McGraw-Hill, New York, NY pp 13–33.
Ecobichon DJ (1990) Chemical management of forest pest epidemics: a case study. Biomed Environ Sci 3:217–239.
Ecobichon DJ (1996) Toxic effects of pesticides. In: Klaassen CD, Amdur MO, Doull J (eds) Casarett & Doull's Toxicology: The Basic Science of Poisons, 5th Ed. McGraw-Hill, New York, NY pp 643–689.
Emerson TE, Jr., Brake CM, Hinshaw LB (1963) Mechanism of action of the insecticide endrin, Report No. FAA-AM-63-16 (1963 Aug). U.S. Department of Transportation, Federal Aviation Agency, Civil Aeromedical Research Institute, Oklahoma City, OK.
Endecott BR, Sanders DC, Chaturvedi AK (1996) Simultaneous gas chromatographic determination of four toxic gases generally present in combustion atmospheres. J Anal Toxicol 20:189–194.
Feldstein M, Klendshoj NC (1957) The determination of volatile substances by microdiffusion analysis. J Forensic Sci 2:39–58.
Felscher D, Wulfmeyer M (1998) A new specific method to detect cyanide in body fluids, especially whole blood, by fluorimetry. J Anal Toxicol 22:363–366.
Fenner OH (1975) Chemical and environmental properties of plastics and elastomers (Chapter 4). In: Harper CA (ed) Handbook of Plastics and Elastomers. McGraw-Hill Book Company, New York, NY pp 4-1–81.
Fowler PR, McKenzie JM (1967) Problems in aerial application: detection of mild poisoning by organophosphorus pesticides using an automated method for cholinesterase activity, Report No. FAA-AM-67-5 (1967 Apr). U.S. Department of Transportation, Federal Aviation Administration, Office of Aviation Medicine, Washington, DC.
Freireich A, Landau D, Dubowski KM, Jain NC (1975) Carbon Monoxide: type B procedure. In: Sunshine I (ed) Methodology for Analytical Toxicology. CRC Press, Inc., Boca Raton, FL pp 67–69.
Fulton HB, Jr. (1985) A pilot's guide to cabin air quality and fire safety. N Y State J Med 85:384–388.
Gallo MA (1996) History and scope of toxicology. In: Klaassen CD, Amdur MO, Doull J (eds) Casarett & Doull's Toxicology: The Basic Science of Poisons, 5th Ed. McGraw-Hill, New York, NY pp 3–11.
Ginestet A, Pugnet D, Rowley J, Bull K, Yeomans H (2005) Development of a new photocatalytic oxidation air filter for aircraft cabin. Indoor Air 15:326–334.
Gossel TA, Bricker JD (1994a) Principles of Clinical Toxicology, 3rd Ed. Raven Press, New York, NY pp 147–178.
Gossel TA, Bricker JD (1994b) Principles of Clinical Toxicology, 3rd Ed. Raven Press, New York, NY pp 109–134.
Griffin BR (1979) A sensitive method for the routine determination of carbon monoxide in blood using flame ionization gas chromatography. J Anal Toxicol 3:102–104.
Guilbault GG, Kramer DN (1966) Ultra sensitive, specific method for cyanide using *p*-nitrobenzaldehyde and *o*-dinitrobenzene. Anal Chem 38:834–836.

Haditsch M (2002) [Flying fitness of patients with infections]. Wien Med Wochenschr 152:469–472.
Harding R (1994) Cabin air quality in aircraft. Bmj 308:427–428.
Harper CA (1975) Fundamentals of plastics and elastomers (Chapter 1). In: Harper CA (ed) Handbook of Plastics and Elastomers. McGraw-Hill Book Company, New York, NY pp 1-1–121.
Hetrick SM, Gould WD, Christensen DE (2000) Inflight cabin ozone aboard long duration C-5 airlift missions: a historical issue revisited. Aviat Space Environ Med 71:408–414.
Hinshaw LB, Emerson TE, Jr., Rieger JA, Jr., Stavinoha WB, Solomon LA, Fiorica V, Reins DA, Holmes DD (1966) Problems in aerial application: I.-V., Report No. FAA-AM-66-11 (1966 Jun). U.S. Department of Transportation, Federal Aviation Administration, Office of Aviation Medicine, Washington, DC.
Hocking MB (1998) Indoor air quality: recommendations relevant to aircraft passenger cabins. Am Ind Hyg Assoc J 59:446–454.
Hocking MB (2000) Passenger aircraft cabin air quality: trends, effects, societal costs, proposals. Chemosphere 41:603–615.
Hocking MB (2002) Trends in cabin air quality of commercial aircraft: industry and passenger perspectives. Rev Environ Health 17:1–49.
ISO International Standard (2008) Analysis of blood for asphyxiant toxicants—carbon monoxide and hydrogen cyanide, Report No. First Edition; ISO 27368:2008 (E) (15 Aug 2008). International Organization for Standardization (ISO), Geneva, Switzerland.
Kuntz DJ, Rao NGS, Berg IE, Khattree R, Chaturvedi AK (1990) Toxicity of mixtures of parathion, toxaphene and/or 2,4-D in mice. J Appl Toxicol 10:257–266.
Lacefield DJ, Roberts PA, Blossom CW (1975) Toxicological findings in fatal civil aviation accidents, fiscal years 1968–1974. Aviat Space Environ Med 46:1030–1032.
Lacefield DJ, Roberts PA, Blossom CW (1978) Agricultural aviation versus other general aviation: toxicological findings in fatal accidents, Report No. FAA-AM-78-31. U.S. Department of Transportation, Federal Aviation Administration, Office of Aviation Medicine, Washington, DC.
Landrock AH (1983) Handbook of Plastics Flammability and Combustion Toxicology: Principles, Materials, Testing, Safety, and Smoke Inhalation Effects. Noyes Publications, Park Ridge, NJ pp 18–22.
Lauwerys RR (1996) Occupational toxicology. In: Klaassen CD, Amdur MO, Doull J (eds) Casarett & Doull's Toxicology: The Basic Science of Poisons, 5th Ed. McGraw-Hill, New York, NY pp 987–1009.
Leder K, Newman D (2005) Respiratory infections during air travel. Intern Med J 35:50–55.
Lewis MF, Mertens HW, Jo Ann Steen BA (1972) Behavioral changes from chronic exposure to pesticides used in aerial application: effects of phosdrin on the performance of monkeys and pigeons on variable interval reinforcement schedules, Report No. FAA-AM-72-29 (1972 Aug). U.S. Department of Transportation, Federal Aviation Administration, Office of Aviation Medicine, Washington, DC.
Lewis RJ, Johnson RD, Canfield DV (2004) An accurate method for the determination of carboxyhemoglobin in postmortem blood using GC-TCD. J Anal Toxicol 28:59–62.
Lindgren T, Norback D (2002) Cabin air quality: indoor pollutants and climate during intercontinental flights with and without tobacco smoking. Indoor Air 12:263–272.
Lindgren T, Norback D (2005) Health and perception of cabin air quality among Swedish commercial airline crew. Indoor Air 15 Suppl 10:65–72.
Lindgren T, Norback D, Andersson K, Dammstrom BG (2000) Cabin environment and perception of cabin air quality among commercial aircrew. Aviat Space Environ Med 71:774–782.
Lindgren T, Norback D, Wieslander G (2007) Perception of cabin air quality in airline crew related to air humidification, on intercontinental flights. Indoor Air 17:204–210.
Loomis TA (1978) Essentials of Toxicology, 3rd Ed. Lea & Febiger, Philadelphia, PA pp 1–12.
McAuley F, Reive DS (1983) Rapid quantitation of cyanide in blood by gas chromatography. J Anal Toxicol 7:213–215.

Meyer E (1977) Chemistry of Hazardous Materials. Prentice-Hall, Inc., Englewood Cliffs, NJ pp 78–99.
Mohler SR, Harper CR (1966) Protecting the AG pilot, Report No. FAA-AM-66-30 (1966 Sep). U.S. Department of Transportation, Federal Aviation Agency, Office of Aviation Medicine, Washington, DC.
Nagda NL, Hodgson M (2001) Low relative humidity and aircraft cabin air quality. Indoor Air 11:200–214.
Nagda NL, Koontz MD (2003) Review of studies on flight attendant health and comfort in airliner cabins. Aviat Space Environ Med 74:101–109.
Nagda NL, Rector HE (2003) A critical review of reported air concentrations of organic compounds in aircraft cabins. Indoor Air 13:292–301.
National Library of Medicine (2008) Aerospace medicine. Retrieved 23 April 2008 from http://www.ncbi.nlm.nih.gov/sites/entrez?db=mesh.
Nicholson AN, Cummin AR, Giangrande PL (2003) The airline passenger: current medical issues. Travel Med Infect Dis 1:94–102.
Odoul M, Fouillet B, Nouri B, Chambon R, Chambon P (1994) Specific determination of cyanide in blood by headspace gas chromatography. J Anal Toxicol 18:205–207.
Pakhomova AA, Mukhamedieva LN, Mikos KN (2006) [Air quality monitoring on the International Space Station]. Aviakosm Ekolog Med 40:46–49.
Patterson RE, Rayman RB (1996) Aerospace air pollution issues. Otolaryngol Head Neck Surg 114:277–280.
Pearson DW, Clark G, Moore CM (1969) A comparison of the behavioral effects of various levels of chronic disulfoton poisoning, Report No. FAA-AM-69-19 (1969 Oct). U.S. Department of Transportation, Federal Aviation Administration, Office of Aviation Medicine, Washington, DC.
Rayman (2001) Aircraft cabin air quality and bills in Congress [editorial: executive director's column]. Aviat Space Environ Med 72:1055.
Rayman (2002) National Academy of Sciences report on cabin air quality [editorial: executive director's column]. Aviat Space Environ Med 73:319.
Rayman RB (1997) Passenger safety, health, and comfort: a review. Aviat Space Environ Med 68:432–440.
Rayman RB (2001) Aircraft cabin air quality: an overview [correction of overview]. Uchu Koku Kankyo Igaku 38:9–15.
Rayman RB (2002) Cabin air quality: an overview. Aviat Space Environ Med 73:211–215.
Rayman RB, McNaughton GB (1983) Smoke/fumes in the cockpit. Aviat Space Environ Med 54:738–740.
Reins DA, Holmes DD, Hinshaw LB (1963) Acute and chronic effects of the insecticide endrin on renal function and renal hemodynamics, Report No. FAA-AM-63-26 (1963 Oct). U.S. Department of Transportation, Federal Aviation Agency, Civil Aeromedical Research Institute, Oklahoma City, OK.
Repace J (2004) Flying the smoky skies: secondhand smoke exposure of flight attendants. Tob Control 13 Suppl 1:i8–19.
Revzin AM (1970) Some acute and chronic effects of endrin on the brain, Report No. FAA-AM-70-11 (1970 Jul). U.S. Department of Transportation, Federal Aviation Administration, Office of Aviation Medicine, Washington, DC.
Revzin AM (1973a) Subtle changes in brain functions produced by single doses of mevinphos (phosdrin), Report No. FAA-AM-73-3 (1973 Feb). U.S. Department of Transportation, Federal Aviation Administration, Office of Aviation Medicine, Washington, DC.
Revzin AM (1973b) Transient blindness due to the combined effects of mevinphos and atropine, Report No. FAA-AM-73-4 (1973 Feb). U.S. Department of Transportation, Federal Aviation Administration, Office of Aviation Medicine, Washington, DC.
Rieders F (1975a) Cyanide: type A procedure. In: Sunshine I (ed) Methodology for Analytical Toxicology, Vol I (5th Printing, 1987). CRC Press, Inc., Boca Raton, FL pp 113–114.
Rieders F (1975b) Cyanide: type B procedure. In: Sunshine I (ed) Methodology for Analytical Toxicology, Vol I (5th Printing, 1987). CRC Press, Inc., Boca Raton, FL pp 114–115.

Rodkey FL, Hill TA, Pitts LL, Robertson RF (1979) Spectrophotometric measurement of carboxyhemoglobin and methemoglobin in blood. Clin Chem 25:1388–1393.

Ryan LC, Endecott BR, Hanneman GD, Smith DR (1970) Effects of an organophosphorus pesticide on reproduction in the rat, Report No. FAA-AM-70-3 (1970 Jan). U.S. Department of Transportation, Federal Aviation Administration, Office of Aviation Medicine, Washington, DC.

Sanders DC (2007) Combustion toxicology research in the Biochemistry Research Unit, CAMI, 1970–1992. In: Collins WE, Wayda ME (eds) Index of FAA Office of Aerospace Medicine Reports: 1961 Through 2006 (DOT/FAA/AM-07/1); 2007 Jan. U.S. Department of Transportation, Federal Aviation Administration, Office of Aerospace Medicine, Washington, DC pp iv–xiv.

Sanders DC, Chaturvedi AK (1994) An overview of aircraft fires, smoke toxicity, and survival – part I. In: Federal Air Surgeon's Medical Bulletin. U.S. Department of Transportation, Federal Aviation Administration, Civil Aeromedical Institute, Oklahoma City, OK p 1 & 6.

Sanders DC, Chaturvedi AK, Hordinsky JR (1999) Melatonin: aeromedical, toxicopharmacological, and analytical aspects. J Anal Toxicol 23:159–167.

Sanders DC, Endecott BR, Chaturvedi AK (1991) Inhalation toxicology: XII. comparison of toxicity rankings of six polymers by lethality and by incapacitation in rats, Report No. DOT/FAA/AM-91/17 (1991 Dec). U.S. Department of Transportation, Federal Aviation Administration, Office of Aviation Medicine, Washington, DC.

Sanders DC, Endecott BR, Chaturvedi AK (1992) Comparison of toxicity rankings of six aircraft cabin polymers by lethality and by incapacitation in rats. Aviat Space Environ Med 63:870–874.

Sanderson JH, Sotheran MF, Stattersfield JP (1978) A new method of carboxyhaemoglobin determination. Br J Ind Med 35:67–72.

Smith PW (1962) Toxic hazards in aerial application, Report No. FAA-AM-62-8 (1962 Apr). U.S. Department of Transportation, Federal Aviation Agency, Civil Aeromedical Research Institute, Oklahoma City, OK.

Smith PW, Robinson CP, Zelenski JD, Endecott BR (1977) The role of monamine oxidase inhibition in the acute toxicity of chlordimeform, Report No. FAA-AM-77-19 (1977 Aug). U.S. Department of Transportation, Federal Aviation Administration, Office of Aviation Medicine, Washington, DC.

Smith PW, Stavinoha WB, Ryan LC (1968) Cholinesterase inhibition in relation to fitness to fly. Aerosp Med 39:754–758.

Smyth KC, Norton TS, Miller JH, Smooke MD (1992) Comparison of experimental and computed species concentration and temperature profiles in laminar, two-dimensional CH4/air diffusion flames. In: Smith SB (ed) Abstracts: Annual Conference on Fire Research (NISTIR 4924), October 13–15, 1992. U.S. Department of Commerce, Technology Administration, National Institute of Standards and Technology, Building and Fire Research Laboratory, Rockville, MD pp 57–58.

Solomon KR, Thompson DG (2003) Ecological risk assessment for aquatic organisms from overwater uses of glyphosate. J Toxicol Environ Health B Crit Rev 6:289–324.

Strahle WC (1993) An Introduction to Combustion. Gordon and Breach Publishers, Amsterdam, Netherlands pp 41–52.

Strom-Tejsen P, Weschler CJ, Wargocki P, Myśków D, Zarzycka J (2008) The influence of ozone on self-evaluation of symptoms in a simulated aircraft cabin. J Expo Sci Environ Epidemiol 18:272–281.

Strom-Tejsen P, Wyon DP, Lagercrantz L, Fang L (2007) Passenger evaluation of the optimum balance between fresh air supply and humidity from 7-h exposures in a simulated aircraft cabin. Indoor Air 17:92–108.

The Encyclopedia Americana (1989) Aviation - The Encyclopedia Americana International Edition. Grolier Incorporated, Danbury, CT p 859.

Thibeault C (1997) Cabin air quality. Aerospace Medical Association. Aviat Space Environ Med 68:80–82.

Thibeault C (2002) Airliner cabin air quality. Occup Med 17:279–292.
Tietz NW, Fiereck EA (1973) The spectrophotometric measurement of carboxyhemoglobin. Ann Clin Lab Sci 3:36–42.
Tracqui A, Raul JS, Géraut A, Berthelon L, Ludes B (2002) Determination of blood cyanide by HPLC-MS. J Anal Toxicol 26:144–148.
U.S. National Aeronautics and Space Administration (1967) Apollo 1: the fire 27 January 1967. Retrieved 14 February 2008 from http://www.history.nasa.gov/SP-4029/Apollo_01a_Summary.htm.
U.S. National Toxicology Program (1999) NTP toxicology and carcinogenesis studies of ethylbenzene (CAS No. 100-41-4) in F344/N rats and B6C3F1 mice (inhalation studies). Natl Toxicol Program Tech Rep Ser 466:1–231.
Uges DRA (2004) Hospital toxicology. In: Moffat AC, Osselton MD, Widdop B, Galichet LY (eds) Clarke's Analysis of Drugs and Poisons in Pharmaceuticals, Body Fluids and Postmortem Material, 3rd Ed. Pharmaceutical Press, London, UK pp 3–36.
Uva Ade S (2002) [Aircraft cabin air quality: exposure to ozone]. Acta Med Port 15:143–151.
van Netten C (1999) Multi-elemental analysis of jet engine lubricating oils and hydraulic fluids and their implication in aircraft air quality incidents. Sci Total Environ 229:125–129.
van Netten C, Leung V (2000) Comparison of the constituents of two jet engine lubricating oils and their volatile pyrolytic degradation products. Appl Occup Environ Hyg 15:277–283.
van Netten C, Leung V (2001) Hydraulic fluids and jet engine oil: pyrolysis and aircraft air quality. Arch Environ Health 56:181–186.
Vieillefond H, Fourn P, Auffret R (1977) Characteristics in the atmosphere of long-range transport aircraft cabins. Aviat Space Environ Med 48:503–507.
Welch B, Navias R (1997) Small fire extinguished on Mir. Retrieved 14 February 2008 from http://www.nasa.gov/centers/johnson/news/releases/1996_1998/97-30.html.
Wick RL, Jr., Irvine LA (1995) The microbiological composition of airliner cabin air. Aviat Space Environ Med 66:220–224.
Widdop B (2002) Analysis of carbon monoxide. Ann Clin Biochem 39:378–391.
Wieslander G, Lindgren T, Norback D, Venge P (2000) Changes in the ocular and nasal signs and symptoms of aircrews in relation to the ban on smoking on intercontinental flights. Scand J Work Environ Health 26:514–522.
Williams LA (1970) Toxicology. In: Frankel S, Reitman S, Sonnenwirth AC (eds) Gradwohl's Clinical Laboratory Methods and Diagnosis, 7th Ed. C.V. Mosby Company, St. Louis, MO pp 303–305.
Williams LA (1975) Carbon Monoxide: type A procedure. In: Sunshine I (ed) Methodology for Analytical Toxicology. CRC Press, Inc., Boca Raton, FL pp 64–66.
Williams LA, Linn RA, Zak B (1960) Ultraviolet absorptiometry of palladium for determination of carbon monoxide hemoglobin. Am J Clin Pathol 34:334–337.
Winek CL, Prex DM (1981) A comparative study of analytical methods to determine postmortem changes in carbon monoxide concentration. Forensic Sci Int 18:181–187.
Wood W, Gabica J, Brown HW, Watson M, Benson WW (1971) Implication of organophosphate pesticide poisoning in the plane crash of a duster pilot. Aerosp Med 42:1111–1113.
Wyss R, Stossel U, Muff S (2001) [Air travel--a disease risk?]. Ther Umsch 58:399–403.
Zamecnik J, Tam J (1987) Cyanide in blood by gas chromatography with NP detector and acetonitrile as internal standard. Application on air accident fire victims. J Anal Toxicol 11:47–48.

Land Application of Sewage Sludge: Physicochemical and Microbial Response

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1 Introduction

As a result of rapid urbanization, industrialization, and uncontrolled population increase, waste management has become a worldwide problem. In the 2001 census, the urban population of India comprised 285 millions, which accounted for 27% of the total population of the country. The share of urban population has increased from 19.9% in the year 1971 to 27.8% in the year 2001 (Vaidya 2009). The decadal growth from 1991 to 2001 of the urban population was 31.2%. One of the main reasons for increasing urbanization is the migration of rural populations to urban centers for employment. The unprecedented growth of this urban population has put tremendous pressure on the quality of life regarding housing, water, and power supply, and water, air, and soil quality deterioration. A decline in environmental quality from waste generation in these urban centers, especially solid waste, is of major and growing concern.

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Solid waste may comprise municipal solid wastes, such as food waste, rubbish, treated waste (industrial and sewage sludge), construction waste, industrial wastes (e.g., chemicals, scrap products, glass, fly ash, resins, industrial sludge, etc.), and hazardous wastes (e.g., volatile organic chemicals and pathological, biomedical, and pharmaceutical wastes). About 48 million tons of municipal solid waste is generated annually in India (Agarwal et al. 2005). Per capita waste generation in major cities of India ranges from 0.2 to 0.6 kg (Devi and Satyanarayana 2001). Urban local bodies spend approximately Rs. 500–1,500 per ton for solid waste collection, transportation, treatment, and its disposal. With increasing urbanization and consequent boost in the urban population, wastewater generation has increased tremendously. Water is the most essential natural resource of Earth, without which life would be impossible. Water resources are becoming increasingly contaminated from anthropogenic activities that result in its pollution. Although water pollution has both human and natural causes, pollution caused by human activities (e.g., industrial and urban effluents, tube well water withdrawal, agricultural runoff, etc.) is generally more widespread. A particularly egregious example of serious human-related pollution is that most sewage generated in towns and cities that are located on the banks of a river is conveniently allowed to flow into the river (Bhargava 2006; Singh and Agrawal 2008).

Stricter regulations on discharge of effluents and sewage into the rivers have been passed and have increasingly resulted in the construction of new sewage-effluent treatment plants. About 22,900 million liters per day (MLD) of domestic wastewater is generated from urban centers, whereas industrial wastewater generation is 13,500 MLD (CPCB 2005). The total treatment capacity in India for domestic wastewater is 5,900 MLD, whereas this value for industrial wastewater is 8,000 MLD. Total wastewater generation, i.e., industrial as well as domestic, has increased from 7,007 MLD in 1978–1979 to 26,254 MLD in 2003–2004, in class I cities (at least 100,000 population – one lakh – and above). However, wastewater treatment capacity has increased from 2,755.94 MLD in 1978–1979 to 7,044 MLD in 2003–2004 (CPCB 2005). Presently, only 26% of total wastewater released from all activities is treated before discharge.

The insoluble solid residue remaining after sewage is finally processed is referred as biosolids, domestic wastewater residuals, or sewage sludge. The prime objective of treatment of wastewater in a sewage treatment plant is to remove pathogens and disinfect effluent prior to its discharge into water bodies. The treatment efficiency of such plants is dependent on the particular treatment processes used. However, removal of solids in the form of sludge, during wastewater treatment, displaces pathogens from the water stream and concentrates them on the sludge solids (Gerba and Smith 2005). Concentration of pathogens in wastewater and sewage sludge is directly associated with an increased incidence of enteric infections at the treatment plant source area of wastewater. Such infections usually result from certain pathogenic forms of *Escherichia coli*, which are ubiquitous and normally exist in the intestines of humans and other vertebrates.

The term biosolids (referring to that solid fraction that remains after sewage treatment) is regarded to emphasize the beneficial nature of this product. The safe disposal of sewage sludge is one of the major environmental challenges throughout the

world. Disposal alternatives frequently undertaken to remove such sludges have included soil application, dumping at sea, land filling, or incineration (Sanchez Monedero et al. 2004). In the United States, dumping of sewage sludge in the ocean was banned as of 31 December 1991 (Hill et al. 1996; USEPA 1999a, b) and in the European Community in 1998 (Zhidong and Wenjing 2009). Land filling and land application of sewage sludge are suggested to be the most economical sludge disposal methods (Mc Grath et al. 1994; Metcalf and Eddy 2003). Land application of sewage sludge is the most economical practice for reducing sewage sludge waste, and this approach also offers the opportunity to recycle beneficial plant nutrients and organic matter to soil for crop production (Laturnus et al. 2007; Singh and Agrawal 2008; Suhadolc et al. 2010; Silva et al. 2010). Assuming that sewage sludge has no significant levels of toxic pollutants, its application on agricultural land has great value, because of the potential it brings for fertilization and soil conditioning. Our purpose in this review is to address the effect that land application of sewage sludge has on soil physicochemical properties and on soil microbial response.

2 Characteristics of Sewage Sludge

The sewage sludges produced at different treatment plants and during different seasons vary in physicochemical properties. As a result, knowledge of the chemical composition of each kind of sewage sludge is necessary before it is used for land application. The characteristic of sewage sludge depends not only on the nature of the wastewater from which it comes but also on the processes by which the wastewater is processed. Sewage sludge is generally composed of organic compounds, macronutrients, a wide range of micronutrients, nonessential trace metals, organic micropollutants, and microorganisms (Kulling et al. 2001; Singh and Agrawal 2008, 2009) (see Table 1). What humans use in their daily lives (e.g., insecticides, detergents, pharmaceuticals, etc.) finds its way to either water or solid waste, and finally reaches treatment plants. Waste from different household industries also contributes heavy metals to waste water and, therefore, ultimately to sewage sludge (Singh and Agrawal 2008). Some important sources of heavy metals that find their way to the wastewater stream and to sewage sludge are shown in Fig. 1.

The macronutrients present in sewage sludge serve as a good source of plant nutrients, and organic constituents impart beneficial soil conditioning properties (Logan and Harrison 1995; Singh and Agrawal 2008). It is very rare that urban sewerage systems transport only domestic sewage to treatment plants. Usually, industrial effluents and storm-water runoff from roads and other paved areas are also discharged into sewerage treatment systems (Singh and Agrawal 2008). Therefore, sewage sludge may contain many different toxic materials (e.g., heavy metals, pesticides, toxic organics, hormone disruptors, detergents, and various salts), in addition to natural organic material (Mc Grath et al. 2000; Singh and Agrawal 2008). Sewage sludge was collected and characterized from eight Indiana cities in the USA over a 2-year period. Results showed that organic N and inorganic

Table 1 Comparison of physicochemical characteristics of sewage sludge from different countries

Properties	Thailand[a]	Spain[b]	India[c]
pH	6.82	8.6	7.0
Electrical Conductivity (ms cm^{-1})			2.28
Organic Carbon (%)	19.82	43.4	5.52
Total Nitrogen (%)	3.43	2.5	1.73
Total Phosphorus (%)	–	1.06	–
Exchangeable K (mg kg^{-1})	870	–	208.96
Exchangeable Ca (mg kg^{-1})	8,332	–	154.13
Total Fe(mg kg^{-1})			6,059
Total Ni (mg kg^{-1})			47.17
Total Mn (mg kg^{-1})			186.2
Total Zn (mg kg^{-1})	801	174	785.3
Total Pb (mg kg^{-1})	1.22	1.00	60.0
Total Cr (mg kg^{-1})	1,326	445	35.5
Total Cd (mg kg^{-1})	2,621	–	154.5

[a]Parkpain et al. (1998), [b]Martinez et al. (2002), [c]Singh and Agrawal (2009)

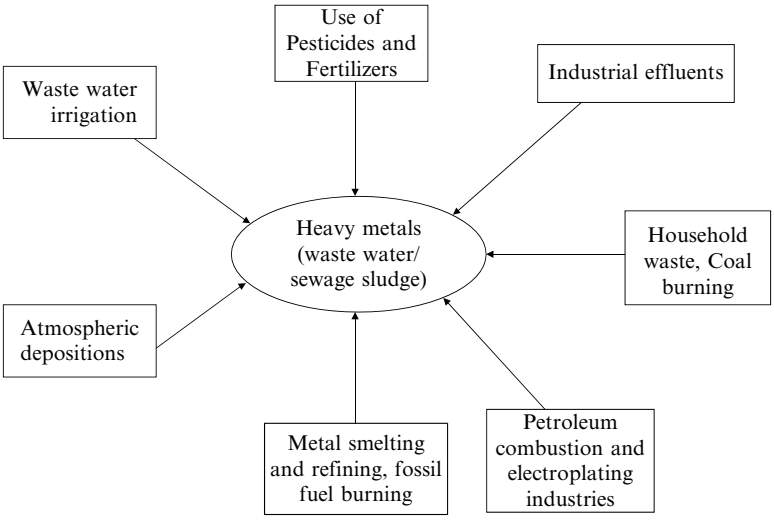

Fig. 1 Sources of heavy metals

P constituted the majority of the total N and P in the characterized sludge, respectively (Sommers et al. 1976). The analyzed sewage sludge contained approximately 50% organic matter and 1–4% inorganic carbon. Relatively constant concentrations of organic and inorganic C, organic N, and inorganic P, Ca, and Mg were present in a given sludge type, throughout the sampling period. The levels of inorganic N, organic P, K, and all other metals were somewhat inconsistent during the entire period of the study (Sommers et al. 1976). The major deviations found were for trace elements and heavy metals, such as Cd, Zn, Cu, Ni, and Pb (Sommers et al. 1976).

Characterization of sewage sludges from Calcutta, India was performed by Maiti et al. (1992) to assess the value of the sludge for plant fertilization. The sewage sludges analyzed were neutral to slightly alkaline and had higher salt content in winter than during the monsoon season (Maiti et al. 1992). Moreover, the cation exchange capacity (CEC) was reported to be higher during the monsoon season. Exchangeable Ca^{+2} was the prevailing cation found in the sludges analyzed, followed by Mg^{+2}, Na^{+}, and K^{+}. The sludges of Calcutta, India were reported to be rich in organic carbon and available N (Maiti et al. 1992).

A comparison of the physicochemical characteristics of sewage sludge collected from different countries is presented in Table 1. Results clearly show that sludge pH varies, and may be either acidic or alkaline (Parkpain et al. 1998; Martinez et al. 2002; Singh and Agrawal 2009). The organic matter content also varied considerably. By contrast, the content of total N and P did not vary that much (Table 1). Among heavy metals present, levels of Cu, Zn, and Mn were variable, whereas the Cd content was more consistent among samples analyzed. The sewage sludge collected from Dindigul (Tamil Nadu), India was recommended for land application, since this sewage sludge had nearly neutral pH, high organic matter, good N, P, and Ca content, and was free of toxic heavy metals such as Cr, Pb, and Hg (Nandakumar et al. 1998).

Singh and Agrawal (2010a, b, c) characterized the sewage sludge from Dinapur Sewage Treatment Plant (DSTP), Varanasi, India. This sewage sludge was neutral in pH and had high electrical conductance and high concentrations of organic C, total N, available P, Fe, Na^{+}, K^{+}, Ca^{2+}, and Mg^{2+} (Singh and Agrawal 2010a, b, c) (Table 1). Zn was present at the highest concentration in this sewage sludge, followed by levels of Cu, Mn, Cd, Pb, Ni, and Cr (Singh and Agrawal 2010a, b, c) (Table 1).

3 Effects of Sewage Sludge Application on Soil Properties

The disposal of sewage sludge by applying it to land is increasing in popularity because of the potential it offers to recycle valuable components (e.g., organic matter, N, P, and other plant nutrients) (Martinez et al. 2002; Singh and Agrawal 2008, 2010b, c). The aapplication of sewage sludge to agricultural soil not only enables nutrients to be recycled, but may eliminate the need for commercial fertilization of cropland (Sommers 1977; Singh and Agrawal 2007, 2009). Because sludges are organic fertilizers, application of them to soils increases soil fertility over time (Archie and Smith 1981). Unwise sewage sludge amendment practices, however, may disturb soil properties, especially when high concentrations of metals and toxic constituents are present in the sludge.

3.1 Physical Properties

The physical condition of soils has been improved by application of sewage sludges (Epstein 1975; Table 2). An increase in soil pH has been reported to occur in soils to which municipal sewage sludge was applied (Tsadilas et al. 1995). Cases of soil

Table 2 Effect of sewage sludge amendments on selected soil physical, chemical, and biological properties

Properties	Effect	References
Physical		
pH	Decrease	Epstein et al. (1976), Nielson et al. (1998), Moreno et al. (1997)
	Increase	Tsadilas et al. (1995), Nielson et al. (1975)
Soil aggregate stability	Increase	Ojeda et al. (2003)
Bulk density	Decrease	Ramulu (2002), Ojeda et al. (2003)
Water-holding capacity	Increase	Epstein (1975), Ramulu (2002)
Porosity	Increase	Ramulu (2002)
Humus content	Increase	Kulling et al. (2001)
Chemical		
Toxic elements	Increase	Adams and Sanders (1984), Kulling et al. (2001), Lopez- Mosaurea et al. (1975)
Soil organic carbon	Increase	Kladivko and Nelson (1975), Singh and Agrawal (2007, 2009, 2010)
Electrical conductance	Increase	Martinez et al. (2002), Ramulu (2002), Singh and Agrawal (2007, 2009, 2010b, c)
N and P	Increase	Martinez et al. (2002), Sommers (1977), Hậni et al. (1996), Walter et al. (2000), Singh and Agrawal (2007, 2009, 2010b, c)
Cation exchange capacity	Increase	Ramulu (2002), Soon (1981)
Biological		
Yeast population	Increase	Kulling et al. (2001)
Pathogenic organisms	Increase	Kulling et al. (2001), Ramulu (2002)
Aerobic bacteria	Increase	Kulling et al. (2001), Ramulu (2002)
Soil microbial activity, soil respiration	Increase	Garcia et al. (1993), Hậni et al. (1996), Banerjee et al. (1997)
	Decrease	Fließbach et al. (1994), Viera and de Souza Silva (2003)

pH being lowered have also been reported after land application of sewage sludges (Epstein et al. 1976; Singh and Agrawal 2010b, c) (Table 2). The changes that occur in soil pH after application of sewage sludges have been correlated with the level of calcium carbonate existing in the sludge, and with production of acids during sludge decomposition (Sommers 1977). Humic acid may be released as a result of biodegradation of sewage sludges rich in organic carbon; such humic acids may contribute to lower soil pH (Moreno et al. 1997). Sorption of metals onto soils is strongly related to soil properties. Generally, heavy metals are more bioavailable for plant uptake at lower pH levels; therefore, the pH of sewage sludge is an important consideration for metal-toxicity potential to plants (Lepp 1981). Several researchers have shown that metal sorption by soils increased with increasing pH (Naidu et al. 1994), organic matter (Gerritse and Van Driel 1984; Udom et al. 2004), cation exchange capacity (Buchter et al. 1989), and the contents of iron and manganese oxides. However, there is a lack of information concerning the adsorption of sludge-borne heavy metals on different soils (Sigua 2005).

Organic matter that is added to soil in the form of sewage sludge composts improves several soil properties, including bulk density, porosity, and water-holding capacity (Ramulu 2002; Table 2). The chemical properties of sludge–soil mixtures not only depend on the properties of soil or sludge or sludge application rates but also on soil pH and on how these components interact (Parkpain et al. 1998). Epstein (1975) conducted a study to evaluate the effect of 0.5% sewage sludge application to soil on water retention, hydraulic conductivity and aggregate stability; results showed that raw and digested sludge improved total soil-water retention capacity, with the greatest enhancement occurring in raw-sludge-amended soil. Moreover, the sludge added to soil resulted in a significant increase in soil hydraulic conductivity after 27 days of incubation. The highest percentage of stable aggregates was reported in a raw sludge treatment that occurred during the first 118 days of incubation. After 175 days, the percentage of stable aggregates for sludge treatments remained the same, averaging 34 vs. 17% for untreated soil (Epstein 1975).

In a field experiment designed to study the effect of long-term sewage sludge application on the chemistry and biology of soils, Hậni et al. (1996) reported increased nutrients (mainly P) and heavy metals from the agricultural use of high levels of sewage sludge. According to Hậni et al. (1996), the soluble fraction of heavy metals, as well organic pollutants in soil, is a determining factor in deciding the stage at which heavy metal toxicity to soil microorganisms or microbial processes in soil is likely to be evident.

3.2 Chemical Properties

According to Hue and Ranjith (1994), the concentrations of metal in sewage sludge depends on factors that include both the (1) origin of the sewage and the (2) sewage treatment processes used. The bioavailability of sludge-borne metals in soil is influenced by several soil properties, including pH, redox potential (Eh), sesquioxide content, and organic matter content, as well as the rate of sludge application (Hue and Ranjith 1994). Adams and Sanders (1984) evaluated the effect pH has on the release rates of Zn, Cu, and Ni from sewage sludges. These authors reported that the concentration of metal released from sewage sludge to the supernatant liquid increased as pH decreased below a threshold value; this threshold value was 5.8 for Zn-loaded sludge, 6.3 for Ni-loaded sludge, and 4.5 for Cu-loaded sludges. The metal content of the supernatant was small and relatively constant at pHs above the aforesaid values. In speciation experiments, the proportion of soluble Cu present as Cu^{+2} in $CuCl_2$ was related to pH, whereas the proportion of soluble Zn present as Zn^{+2} was scarcely correlated with pH (Adams and Sanders 1984).

Hernandez et al. (1991) conducted a study to analyze what influence sewage sludge application had to a Calciorthid soil on the soil availability of macronutrients (N, P, and K) and heavy metals (Fe, Cu, Zn, Mn, Ni, Cr, Cd, and Pb). The total N and extractable N and P content increased in the sludge-amended soil, whereas the extractable K remained unaltered. The Cu, Zn, and Pb levels increased, while Fe

content decreased. Extractability of Fe, Cu, Mn, Zn, and Pb increased, when sludge was applied, as compared to the unamended control.

Relatively high rates of sludge application increased the soil cation exchange capacity, which helped to retain essential plant nutrients within the rooting zone. Such nutrients are retained as a result of additional cation binding sites being created (Soon 1981). Such responses, however, depend upon the sewage soil ratio. The higher the proportion of organic matter in sludges, the more bulk density was decreased and aggregate stability increased (Ojeda et al. 2003; Table 2). Sludge-related improvements in soil physical properties also increased water-holding capacity by promoting higher water retention in sludge-amended soils (Ojeda et al. 2003; Table 2).

Analysis of sewage-sludge-fed agricultural soil layers (0–15 and 15–30 cm) around Calcutta, collected from different upland and lowland sites, showed slightly alkaline pHs (Maiti et al. 1992). Subsurface soil had a higher pH (7.5) than did the surface ones (7.3). The soil of upland sites had slightly higher CEC [18.4–22.8 (cmol (p^+ kg^{-1}))] than did lowland ones [15.1–19.1 (cmol (p^+ kg^{-1}))]. Surface soil contained higher amounts of organic carbon (1.31%) than did subsurface ones (1.16%). Ca^{+2} was the dominant cation [11.5–19.3 cmol (p^+ kg^{-1})] in the sewage-fed soil, followed by Mg^{+2} [2.1–2.7 cmol (p^+ kg^{-1})], Na^+ [0.4–0.9 cmol (p^+ kg^{-1})], and K^+ [0.1–0.3 cmol (p^+ kg^{-1})]. Available N and P were at moderate to high levels.

Sewage sludge amendment always poses an environment risk, resulting from nutrient imbalances and toxic element accumulation, and leaching. Transfer of metal from sewage sludge to soil and later to groundwater via leaching poses potential health and environmental risks from plant uptake (McBride et al. 1997; Bhogal et al. 2003; Mahdavi and Jafari 2010). Korboulewsky et al. (2002) studied the effects of sewage sludge composts applied at the rates of 10, 30, and 90 tons ha^{-1} fresh wt, on a vineyard in southeastern France. These authors quantified the rate of in situ N mineralization, soil organic matter levels, and evaluated selected environmental risks, including N and P leaching rates, and levels of heavy-metal accumulation in soil. It was found that soil organic matter levels increased at all the treatment doses, but neither total nor available heavy metal concentrations increased. Because the sewage sludge studied contained very low levels of heavy metals, and existed mainly in nonextractable and nonexchangeable forms (Breslin 1999), composting it reduced the heavy metal availability by adsorption or complexing processes with humic substances. The levels of mineral nitrogen present increased in the plots in which the topsoil was amended during the first and the second summers. The risk of N leaching was very low in contrast to P at the recommended sludge amendment rate. The increase of P content in amended soil was significant in both top and subsoil layers in all treated plots. The maximum increase in P content occurred at the highest rate of sludge applied. However, at lower sludge amendment rates no significant differences were observed. It has been shown, in column leaching (Ashworth and Alloway 2004) studies, and in batch (Burton et al. 2003) experiments, that heavy metal ions may leach more easily in the presence of sewage sludge than in its absence. Moreover, as dissolved organic matter (DOM) concentration increases, the movement and translocation of heavy metals in soil increases; by contrast, an

increase in soil organic matter (bounded with soil particles) and pH decreases heavy metal mobility (Liu et al. 2007; McCarthy and Zachara 1989; McBride et al. 1999). As a result of the net negative charge of DOM at typical soil pHs, it generally moves easily through the soil system (Dunnivant et al. 1992).

Magdoff and Amadon (1980) performed both laboratory and field experiments to evaluate the contribution that the nitrogen in sewage sludge makes to crops. Aerobically treated secondary liquid sewage sludge was applied to supply 50, 100, 150, and 200 kg N ha^{-1} $year^{-1}$ (as ammonium nitrate) to corn (*Zea mays L.*) and hay (timothy, Kentucky bluegrass, quack grass, and red and white clover). These forages were grown on Hadley sandy loam and Nellis loam soils. Under laboratory conditions, more than 54% of the organic N added to the sludge-amended corn soil was mineralized. Under field condition, mineralization of organic N from sludge that had been applied to corn and hay averaged 55% during the first year of application. The amount of sludge organic N mineralized appeared to vary according to the percent organic N present in the sludge (Sommers 1977).

In soil, trace elements may exist as solid phases, free ions in soil solution, soluble organic mineral complexes, or adsorbed onto colloidal particles. Addition of sewage sludge to soils may affect the potential availability of heavy metals to plants (Wang et al. 1997). The solubility, and consequently the mobility of metals added in sewage sludge are controlled, in part, by organic matter decomposition and resultant creation of soluble organic carriers of metals (Chaney and Ryan 1993). Trace metal bioavailability is also dependent upon the form of organic matter present, i.e., soluble (fulvic acid) or insoluble (humic acid) forms (McBride 1995). Insoluble organic matter inhibits the uptake of metals, which are tightly bound to organic matter, thus reducing bioavailability. Soluble organic matter, however, increases bioavailability by forming soluble metal organic complexes (Mc Bride 1995). When organic matter decomposition rates are stable, the level of soluble organic matter present is reduced, which leads to a reduction in metal bioavailability.

Morera et al. (2002) studied the bioavailability of Cu, Ni, Pb, and Zn from municipal sewage sludge to sunflower plants (*Helianthus annus L.*) in four different types of soils (i.e., Ithnic Haplumbrept (LH), Calcixerollic Xerochrept (Cx1 and Cx2), and Paralithic Xerorth (Px)). Each of these soils retained different physicochemical properties. The purpose of the experiment was to evaluate the influence of several sewage sludge application rates (0, 80, 160, and 320 tons/ha dry wt.), and soil type on the bioavailability of heavy metals, and interaction among these variables. The acid pH of the LH soil favored the bioavailability of Zn from sewage sludge, whereas Cu bioavailability was greater in alkaline soils. The high organic matter content of the acid soil (LH) produced complexes with Cu and thus impaired its uptake by plants. A contrasting trend occurred with respect to metal concentrations in acid and alkaline soils. The plant concentrations of Zn, Cu, Pb, and Ni decreased with increases in sludge application rates for the acid soil (LH), whereas in alkaline soils (Cx1), Zn and Cu levels increased. There were minor changes in metal concentrations of plants grown in Cx2 and Px soils that resulted from increased sludge doses. The results of this study further suggested that soil type has a larger effect on metal bioavailability than did sludge application rates.

3.3 Biological Properties

Species used to monitor the health of an environment or ecosystem are referred to as "biological indicators." Biological indicators may constitute any biological species or group thereof, that have a function or population marker that can be used to determine ecosystem or environmental integrity. Biological indicators are often employed to represent some aspect of living soil components, and such indicators usually respond more rapidly than do physical and chemical indicators to changing soil conditions (Anderson and Gray 1990; Powlson 1994; Pascual et al. 2000). Moreover, biological indicators are useful as sensitive tools for detecting changes in soil conditions that may occur.

3.3.1 Soil Microbial Biomass and Enzymes

Of total soil microbial biomass, soil fungi often comprise at least 75–95% and together with bacteria are responsible for about 90% of the total energy flux of organic matter decomposition in soil (Paul and Clark 1996). Among the key fertility parameters and biological properties of soils, special emphases are given to soil enzyme activity.

Soil enzymatic measurements are used to provide a biological index of soil fertility, and soil enzyme activity is used as an indicator for many soil biological processes. Soil enzymatic activities have often been used to establish indices of soil fertility, since they reflect the effects of cultivation, soil properties, and pedological amendments (Skujins 1978; Ceccanti et al. 1993). Soil enzymes are constantly being synthesized, accumulated, inactivated, and/or degraded and, therefore, play vital agricultural and nutrient cycling roles (Tabatabai 1994; Dick 1997). The heavy metals present in sewage sludge may indirectly affect soil enzymatic activities (Kandeler et al. 2000). According to Fließbach et al. (1994), the effect of sewage sludge on biological activity can be used as a soil pollution indicator. Amending soils with sewage sludge increased soil microbial activity, soil respiration, and soil enzymatic activities (Banerjee et al. 1997). However, when incubations were longer and heavy metal availability was higher, reduced soil enzyme activities were reported (Fließbach et al. 1994).

Urease (urea amidohydrolase) is the enzyme that catalyzes the hydrolysis of urea to CO_2 and NH_4 ions, by acting on C-N nonpeptide bonds in linear amides (Antonious 2009). Urease is an important soil enzyme that also mediates the conversion of organic N to inorganic N by hydrolysis of urea to ammonia (Byrnes and Freney 1995). Invertase (β-D-fructofuranosidase) is a ubiquitous enzyme in soils (Gianfreda et al. 1995). The activities of urease and invertase are important in soil for releasing simple carbon and nitrogen sources that contribute to the growth and multiplication of soil microorganisms. According to Garcia et al. (1993), sewage sludge contains high amounts of enzymatic substrates. These easily available substrates stimulate microbial growth and enzyme production. Suhadolc et al. (2004), pointed out that

increased Pb, Zn, and Cd bioavailability in heavy-metal-contaminated soils affects the structure of soil microbial communities and significantly reduces the rate of mineralization of the pesticide isoproturon (from 20 to 5%).

The effects of adding different levels (0, 100, 200, and 300 ton ha^{-1} dry wt.) and C/N ratios (3:1, 6:1, and 9:1) of sewage sludge on activities of β-glucosidase, alkaline phosphatase, arylsulphatase, and urease in a clay loam soil (at 25°C and 60% water-holding capacity) were studied by Kizilkaya and Bayrakli (2005). Nitrogen was added to the sludge as a $(NH_4)_2SO_4$ solution to obtain the desired C/N ratios. Compared to unamended control samples, a more rapid and significant increase in soil enzymatic activity occurred at different doses and at different C/N ratios of the sewage sludge amendments. Enzyme activities varied with differences in incubation period. Soils with the highest C/N ratio and sludge dose had the highest ß-glucosidase activity. Alkaline phosphatase and aryl sulphatase showed an incremental increase in their activity during the first 30 days of incubation, followed by a pronounced decrease as compared to unamended soil. Urease activity, however, showed an increase within 15 days, and thereafter its activity declined. The highest activities of urease, alkaline phosphatase, and arylsulphatase were observed in soil amended with a low C/N ratio and the highest dose of sludge.

Parat et al. (2005) studied the long-term (20 year) effect of farm yard manure (FYM) (10 ton ha^{-1} $year^{-1}$) and sewage sludge (10 and 100 ton ha^{-1} $year^{-1}$, added every 2 years) amendments on soil organic matter in a fluvisol soil. At the highest dose of sludge amendment, the organic carbon content was 2.5 times higher than that of the unamended soil. Microbial biomass also remained higher in the sludge-amended soils. In another study, three different soil types were treated with two sources of sewage sludge at four different rates (1, 3, 10, and 20% sludge/soil ratio dry wt.), and the β-glucosidase activity at different incubation periods was assayed (Eivazi and Zakaria 1993). Enzyme activity was inhibited at the lower loadings, but was enhanced at the higher application rates. Inhibition of enzyme activity was more pronounced for the soil having higher trace-metal concentrations in the sludge (Eivazi and Zakaria 1993). The increase in enzyme activity was attributed to enhanced microbial activity, which was stimulated by the higher nutrient and organic matter content levels of the sludge-amended soil.

Hậni et al. (1996) reported that the microbial activity in soil will normally be boosted when sewage sludge amendments are added. But, immediate enrichment with organic matter or inorganic and organic pollutants has also produced negative effects on soil microflora. The most significant harmful effects produced on soil microorganisms from sludge applications are the reduced size of total biomass, reduced nitrogen fixing activity, and changes in soil microbial population composition. However, it is still uncertain at which step heavy-metal toxicity becomes evident to soil microorganisms or to microbial processes (Hậni et al. (1996).

Viera and de Souza Silva (2003) studied the effect of frequent sludge amendments on soil dehydrogenase activity and microbial biomass C. The experiment was carried out at Jaguariuna, Brazil on loamy/clayey-textured dark red dystroferric oxisol, in which maize was cultivated. The treatments included: a control without fertilization or sludge, a chemical fertilized treatment, a sludge dose 1 (1N), sludge

dose 2 (2N), sludge dose 3 (4N), and sludge dose 4 (8N). The dose of sludge was based on an N concentration equivalent to that recommended for maize. Other doses were two, four and eight times the base dose. The sludge was applied in April 1999, December 1999, and October 2000. After 132 days of incubation, the sludge dose 8N was detrimental to the soil microflora, as a result of the soil microbial biomass and dehydrogenase activity being reduced at this amendment dose.

Microbial populations and enzymatic activities in sewage-sludge-amended soils were studied at two application rates (5 and 1% dry wt.) by Hattori (1988) to elucidate the role of soil microorganisms in decomposing sewage sludge. The authors found that organic C and N mineralization rates rapidly increased bacterial number and proteinase activity in the soil and reached a maximum within the first 3 days, declining rapidly thereafter. The actinomycetes and fungi counts reached their maximum after 2 or 3 weeks of incubation and thereafter remained at the same level. The amino-acid N content found in 6N HCl extracts of sludge-amended soil decreased markedly. The proteinase-producing bacteria contributed significantly to the rapid degradation observed during the early days of the sludge amendment experiment, whereas actinomycetes and fungi contributed to a gradual degradation during the end phase. The sludge amendment enhanced soil microbial biomass by 8–28% (at the sludge amendment rate of 0.75% dry wt.), and the enhancement was greatest in the clay-loam, and the least in the sandy-loam soil (Dar 1996). The activities of three soil enzymes (i.e., dehydrogenase, alkaline phosphatase, and arginine ammonification) were enhanced by 18–25%, 9–23% and 8–12%, respectively, as compared to activities in unamended soils. The increase was greater in sandy loam than in loam, or clay loam soils.

Soil fertility may increase from additions of sewage sludge, although sludges may also be important causes of soil pollution. Some metals present in sludge, e.g., Cu, Ni, and Zn, are essential micronutrients for plants and microorganisms (Alloway 1995). However, at higher concentrations, even these micronutrients may be toxic. Adverse effects of sludge metals on soil microorganisms pose a potential threat to soil quality, particularly through the disruption of nutrient cycling. In most studies in which soils have been amended with sewage sludge, reductions in microbial biomass (Leita et al. 1995; Fließbach et al. 1994) and enzymatic activity (Kuperman and Carriero 1997) were found, when soils were contaminated with heavy metals. However, the influence of heavy metals on soil respiration is less well known. Some researchers have reported significantly lower CO_2 evolution in metal-contaminated soils (Doelman and Haanstra 1984; Freedman and Hutchinson 1980; Hattori 1992, Kuperman and Carreiro 1997). Others have reported the opposite (e.g., Leita et al. 1995; Fließbach et al. 1994; Bardgett and Saggar 1994). Additionally, a range of studies have indicated that respiration responses to metal inputs may vary with the time that has elapsed since application (e.g., Doelman and Haanstra 1984). Sludge applied to soils often contains a variety of metals. Responses of microbes to such metal combinations may be synergistic, antagonistic, or additive (Chander and Brookes 1991a). Because of the complexity of such interactions, it is very difficult to establish a minimum soil concentration for individual metals at which adverse effects on microorganisms may occur (Brookes 1995). Chander and

Table 3 Principal pathogens of concern in municipal wastewater and sewage sludge

Pathogen of concern	Disease or symptoms caused by the organism
Bacteria	
Salmonella spp.	Salmonellosis (food poisoning), typhoid
Shigella spp.	Bacillary dysentery
Yersinia spp.	Acute gastroenteritis (diarrhea, abdominal pain)
Vibrio cholerae	Cholera
Campylobacter jejuni	Gastroenteritis
Escherichia coli	Gastroenteritis
Viruses	
Poliovirus	Poliomyelitis
Coxsackievirus	Meningitis, pneumonia, hepatitis, fever
Echovirus	Meningitis, paralysis, encephalitis fever
Hepatitis A virus	Infectious hepatitis
Reovirus	Respiratory infections, gastroenteritis
Astroviruses	Gastroenteritis
Protozoa	
Cryptosporidium	Gastroenteritis, cryptosporidiosis
Entamoeba histolytica	Acute enteritis
Giardia lamblia	Giardiasis (diarrhea and abdominal cramps)
Toxoplasma gondii	Toxoplasmosis
Balantidium coli	Diarrhea, dysentery
Helminth worms	
Ascaris lumbricoides	Digestive disturbances, abdominal pain
Trichuris trichiura	Diarrhea, anemia, weight loss
Taenia sasginata	Nervousness, insomnia, anorexia
Taenia solium	Nervousness, insomnia, anorexia
Hymenolepis nana	Taeniasis
Necator americanus	Hookworm disease

Source: Rose et al. (1996); Epstein (1998); USEPA (1999a, b)

Brookes (1991b) reported that, in metal contaminated soils, microorganisms are under stress and soil biomass reduction occurs mainly as a result of inefficient biomass synthesis.

3.3.2 Pathogens

According to Sidhu et al. (2001), biosolids originating from wastewater treatment plants contain a wide range of pathogens naturally, some of which may be present in large numbers and may represent a public health hazard (e.g., *Salmonella* spp.). In dehydrated anaerobically digested sludge wastewater, the concentration of *Salmonella* spp. may exceed 10^5 g^{-1} of dry wt. (Russ and Yanko 1981). According to Epstein (1998), some pathogens are found in sewage sludge and these long survive their land application (Table 3). Viruses of small size and other pathogens present in sewage

sludge, if not killed, may leach into ground water (Powelson et al. 1991). There are four major types of human pathogenic (disease-causing) organisms (bacteria, viruses, protozoa, and helminthes), and all may be present in domestic sewage. The actual species and quantity of pathogens present in domestic sewage from a particular municipality depends on the health status of the local community, and may vary significantly at different times. The concentration of pathogens in treated sewage sludge (biosolids) also depends on the reductions achieved by wastewater and sewage-sludge treatment processes. During the typical wastewater treatment process, the microorganisms present in sewage are reduced in number and become concentrated in the sewage sludge. However, some pathogens are still present in the effluent, which can contaminate recreational waters and drinking water supplies (Rose et al. 1996) (Table 3).

Lewis and Gattie (2002) reported that, of all the pathogenic organisms present in sewage sludge, enteric viruses are of the utmost risk to humans, owing to their resistance to high pH and heat treatment, high infectivity, and survivability (Gibbs et al. 1994; Lewis and Gattie 2002). The bacteria (e.g., fecal coliform, *Listeria monocytogenes*, and enterococci) found in sludge are capable of surviving anaerobic digestion (Sidhu 2000; Gerba et al. 2002; Estrada et al. 2004). After waste treatment processes and land application of sewage sludge, regrowth may also occur (and does occur with *Salmonella* sp). Several plant disease-causing pathogens have also been reported to exist in sewage sludge (Santos and Bettiol 2003; Al-Zubeiry 2005). Contamination of ground and surface water by chemicals and pathogens, and odor from volatile organics are some of the potential problems associated with the use of sludges on cropland (USEPA 1994).

Bioaerosols are particulate matter of microbial, plant or animal origin that measure less than 20 μm in diameter (Goyer et al. 2001). They consist of pathogenic or nonpathogenic live or dead bacteria, viruses, molds, pollens, etc. Bioaerosols are of considerable concern because they are associated with a wide range of health problems such as contagious infections, allergies, and cancer (Bray and Ryan 1991; Douwes et al. 2003). These are also of concern from the use of sewage sludge amendment, since bioaerosols can transmit many enteric microorganisms (Pahren and Jakubowski 1980). Land application of sewage sludge can result in the transport of pathogens through aerosols downwind of sludge storage sites, contamination of ground water, drinking-water wells, stock ponds, or food chain contamination from eating food grown in sludge-treated land.

4 Conclusions

The land application of sewage sludges to agricultural soils, and associated practices, is the most cost-effective management technique for disposing of sewage sludges and offers potential improvements over conventional disposal methods, such as landfilling or incineration. One advantage of such land application of sludges is that it recycles plants nutrients that are present in the sludge. Nutrients present in sewage sludge are also useful to the soil microbial biomass. Using sewage sludge

as a soil amendment promotes microbiological activity, but may have the opposite outcome if toxic heavy metals are present in the applied sludges. Researchers have studied both the positive and negative effects of land application of sewage sludges and have reported dissimilar effects from sewage sludge on soil microbial biomass and activity. In some studies, appreciable concentrations of heavy metals in sewage sludge did not appear to have any negative influence on soil microbial biomass and enzyme activities. However, other reports have clearly illustrated that the heavy metals present in sewage sludge do decrease the proportion of microbial biomass C in total soil organic matter.

Increases in soil microbial biomass from sewage sludge amendments mainly result from stimulation of the indigenous soil microbes by microbes present in the sludge organic residues. Microbial biomass also increases from the addition of substrate-C. The effect of heavy metals on soil microbes depends on the characteristics of the soil as well as on the character and rates of sewage sludge applied. It is known that that biosolids originating from wastewater treatment plants contain a wide variety of pathogens. Several plant-disease causing pathogens have also been reported to occur in the biosolids.

Our main conclusions from reviewing the cogent literature and from preparing this review are as follows:

1. Although the application of sewage sludge in agricultural practice may be beneficial, it also may contaminate ground water, drinking water from wells, and the food chain
2. Land applications of sewage sludge may result in transport of pathogens through aerosols to areas of human habitation
3. Considering the foregoing, the physicochemical analysis of sewage sludge is necessary before a decision is made to use it for land application
4. To reach a clearer conclusion on the value of sewage sludge disposal by land application under diverse conditions, more research is required. In particular, research is needed on application to different soil types and at sewage sludge amendment rates to evaluate effects on soil microbial biomass

5 Summary

In the present review, we address the effects of sewage sludge amendment on soil physicochemical properties and on soil microbial biomass. Sewage sludge is a by-product of sewage treatment processes and is increasingly applied to agricultural lands as a source of fertilizer, and as an alternative to conventional means of disposal. The particular characteristics of sewage sludge depend upon the quality of sewage from which it is made, and the type of treatment processes through which it passes. Sewage sludge may substitute for inorganic fertilizers because it is rich in organic and inorganic plant nutrients.

However, the presence of potentially toxic metals and pathogens in sewage sludge often restricts its uses. Ground water and food chain contamination resulting

from sewage sludge amendment is one major concern worldwide. The health of soils is represented by a composite of their physical, chemical and biological properties. Amending soil with sewage sludge modifies the physicochemical and biological properties of soils. Perhaps the central constituent of soil that is important in the context of sewage sludge amendment is microbial biomass. Soil microbial biomass, the key living part of the soil, is very closely associated with the content of organic matter that exists in arable agricultural soils. When sewage sludge is land-applied, soil enzyme activities may be directly or indirectly affected by the presence of heavy metals. In several studies, results have shown that sewage sludge amendment increased soil microbial and soil enzyme activities; however, reduction in soil enzyme activity has also been reported. When incubation periods of sewage sludge were longer, heavy metal bioavailability increased. Soil pathogenic activity has also been reported to increase as a result of land application of sewage sludges. The level of pathogens in treated sewage sludge (biosolids) depends on the processes used to treat wastewater and sewage sludge. Agricultural application of sewage sludge may result in the transport of pathogens through aerosols downwind of sludge storage or dispersal sites, may contaminate ground water, stock ponds, or may produce food chain contamination from eating food grown in sludge-treated land.

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References

Adams TMM, Sanders JR (1984) The effect of pH on the release to solution of zinc, copper and nickel from metal loaded sewage sludges. Environ Pollut 8:85–99.

Agarwal A, Singhmar A, Kulshrestha M, Mittal AK (2005) Municipal solid waste recycling and associated markets in Delhi, India. Resour Conserv Recy 44:73–90.

Alloway BJ (1995) Heavy metals in soils, Blackie Academic and Professional. pp 11–37.

Al-Zubeiry AHS (2005) Microflora inhabiting raw sewage, secondary effluent and dewatered sludge in IBB, Yemen republic Ass. Univ Bull Environ Res 8 No. 1

Anderson TH, Gray TRG (1990) Soil microbial carbon uptake characteristics in relation to soil management. FEMS Microbiol Ecol 74:11–19.

Antonious GF (2009) Enzyme activities and heavy metals concentration in soil amended with sewage sludge. J Environ Sci Health A 44:1019–1024.

Archie SG, Smith M (1981) Survival and growth of plantations in sewage sludge treated soil and older forest growth study. In municipal sludge application to Pacific North-West forest lands. Bledose CB (ed) pp105–113 Univ of Washington, College of Forest Resources, Washington, D.C.

Ashworth D J, Alloway B J (2004) Soil mobility of sewage sludge-derived organic matter, copper, nickel and zinc. Environ Pollut 127:37–144.

Banerjee MR, Burtonand DL, Depoe S (1997) Impact of sewage sludge application on soil application characteristics. Agric Ecosyst Environ 66:241–249.

Bardgett RD, Saggar S (1994) Effects of heavy metal contamination on the short-term decomposition of labelled [14C] glucose in a pasture soil. Soil Biol Biochem 26:727–733.

Bhargava D (2006) Revival of Mathura's ailing Yamuna river. The Environmentalist. 26:111–122, DOI: 10.1007/s10669-006-7481-1

Bhogal A, Nicholson FA, Chambers BJ, Shepherd MA (2003) Effects of past sewage sludge additions on heavy metals availability in light textured soils: implications for crop yields and metal uptakes. Environ Pollut121:413–423.

Bray GA, Ryan DH (eds) (1991) Mycotoxins, cancer, and health. Baton Rouge, LA: Louisiana State University Press.

Breslin VT (1999) Retention of metals in agricultural soils after amending with MSW and MSW-biosolids compost. Water Air Soil Pollut 109:163–178.

Brookes PC (1995) The use of microbial parameters in monitoring soil pollution by heavy metals. Biol Fer Soils 19:269–279.

Buchter B, Davidoff B, Amacher MC, Hinz C, Iskandar I K, Selim H M (1989) Correlation of Freundlich Kd, and retention parameters with soils and elements. Soil Sci 148:370–381.

Burton ED, Hawker DW, Redding M R (2003) Estimating sludge loadings to land based on trace metal sorption in soil: effect of dissolved organometallic complexes. Water Res 37: 1394–1400.

Byrnes BH, Freney JR (1995) Recent development on the use of urease inhibitors in the tropics. Fertilizer Res 42:251–259 .

Ceccanti B, Pezzarossa B, Gallardo-Lancho FJ, Masciandaro G (1993) Biotests as markers of soil utilization and fertility. Geomicrobiol J 11:309–316.

Central pollution control board (CPCB) (2005) Sewage Pollution -February 2005.

Chander K, Brookes PC (1991a) Effects of heavy metals from past applications of sewage sludge on microbial biomass and organic matter accumulation in a sandy loam and silty loam UK soil. Soil Biol Biochem 23:927–932.

Chander K, Brookes PC (1991b) Microbial biomass dynamics during the decomposition of glucose and maize in metal-contaminated and non-contaminated soils. Soil Biol Biochem 23:917–925.

Chaney RL, Ryan JA (1993) Heavy metals and toxic organic pollutants in MSW-composts: Research results on phytoavailability, bioavailability, etc. pp 451–506. In: Hoitink HAJ and Keener HM (eds) Science and Engineering of Composting: Design, Environmental, Microbiological and Utilization Aspects. Ohio State Univ. Columbus, OH.

Dar GH (1996) Effects of Cadmium and sewage sludge on soil microbial biomass and enzymes activities. Bioresour Technol 56:141–145.

Devi K, Satyanarayana V (2001) Financial resources and private sector participation in SWM in India. Indo-US Financial Reform and Expansion (FIRE) Project, New Delhi.

Dick RP (1997) Soil enzyme activities as integrative indicators of soil health. In: Pankhurst CE, Doube BM, Gupta VVSR (eds) Biological Indicators of Soil Health, CAB International, Wellingford pp 121–156.

Doelman P, Haanstra L (1984) Short-term and long-term effects of cadmium, chromium, copper, nickel, lead and zinc on soil microbial respiration in relation to abiotic soil factors. Plant Soil 79:317–327.

Douwes J, Thorne P, Pearce N, Heederik D (2003) Bioaerosol Health Effects and Exposure Assessment:Progress and Prospects. Ann Occup Hyg 47:187–200.

Dunnivant FM, Jardine PM, Taylor DL, McCarthy JF (1992) Transport of naturally occurring dissolved organic carbon in laboratory columns containing aquifer material. Soil Sci Soc Am J 56:437–444.

Eivazi F, Zakaria A (1993) ß-glucosidase activity in soils amended with sewage sludge. Agric Ecosyst Environ 43:155–161.

Epstein E (1975) Effect of sewage sludge on some soil physical properties. J Environ Qual 4: 139–142.

Epstein E (1998) Pathogenic health aspects of land application. Biocycle 39:62–66.

Epstein E, Taylor JM, Chaney RL (1976) Effects of sewage sludge and sludge compost applied to soil on some soil physical and chemical properties. J Environ Qual 5:422–26.

Estrada A, Aller A, Aller F, Gomez X, Moran A (2004) 'The survival of *Escherichia coli*, faecal coliforms and enterobacteriaceae in general in soil treated with sludge from waste water treatment plants. Bioresour Technol 1–8.

Fließbach A, Martens R, Reber HH (1994) Soil microbial biomass and microbial activity in soils treated with heavy metal contaminated sewage sludge. Soil Biol Biochem 26:1201–1205.
Freedman B, Hutchinson TC (1980) Effects of smelter pollutants on forest leaf litter decomposition near nickel-copper smelter at Sudbury, Ontario. Can J Bot 58:1722–1736
Garcia C, Hernandez T, Costa C, Ceccanti B, Masciandaro G, Ciardi C (1993) A study of biochemical parameters of composted and fresh municipal wastes. Bioresour Technol 44:17–23.
Gerba CP, Pepper IL, Whitehead LF (2002) A risk assessment of emerging pathogens of concern in the land application of biosolids. Water Sci Tech 46:225–230.
Gerba CP, Smith JE (2005) Sources of pathogenic microorganisms and their fate during land application of wastes. J Environ Qual 34:42–48.
Gerritse RG, Van Driel W (1984) The relationship between adsorption of trace metals, organic matter and pH in temperate soils. J Environ Qual 13:197–204.
Gianfreda L, Sannino F, Violante A (1995) Pesticide effect on the activity of free, immobilized and soil invertase. Soil Biol Biochem 27:1201–1208.
Gibbs R A, Hu C J, Ho G E, Unkovich I, Phillips P A (1994) Die-off of Human Pathogens in Stored Wastewater Sludge and Sludge Applied to Land, Water Authority of WA, Perth, WA.
Goyer N, Lavoie J, Lazure L, Marchand G (2001) Les bioaérosols en milieu de travail: guide d'évaluation, de contrôle et de prévention. Études et recherches, IRSST, Technical Guide T-23., Montreal, Sept. 2001. 57 p.
Hâni H, Siegenthaler A, Candinas T (1996) Soil effects due to sewage sludge application in agriculture. Nutr Cycl Agroecosys 43: 149–156, DOI: 10.1007/BF00747695.
Hattori H (1988) Microbial activities in soil amended with sewage sludges. Soil Sci Plant Nutr 34: 221–232.
Hattori H (1992) Influence of heavy metals on soil microbial activities. Soil Sci Plant Nutr 38: 93–100.
Hernandez T, Moreno JI, Costa F (1991) Influence of sewage sludge application on crop yields and heavy metal availability. Soil Sci Plant Nutr 37:201–210.
Hill RT, Straube WL, Palmisano AC, Gibson SL, Colwell RR (1996) Distribution of sewage indicated by Clostridium per fringes at a deep-water disposal site after cessation of sewage disposal. Appl Environ Microbiol 62:1741–1746.
Hue NV, Ranjith SA (1994) Sewage sludges in Hawaii: Chemical composition and reactions with soils and plants. Water Air Soil Pollut 72:265–283.
Kandeler ED, Tscherko KD, Bruce M, Stemmer PJ, Hobbs RD, Bardgett W, Amelung (2000) Structure and function of the soil microbial community in microhabitats of a heavy metal polluted soil. Biol Fertil Soils 32:390–400.
Kizilkaya R, Bayrakli B (2005) Effects of N-enriched sewage sludge on soil enzyme activities. Appl Soil Ecol 30:192–202.
Kladivko EJ, Nelson DW (1979) Changes in soil properties from application of anaerobic sludge. J Water Pollut Control Fed 51:325–332.
Korboulewsky N, Dupouyet S, Bonin G (2002) Environmental risks of applying sewage sludge compost to vineyards: Carbon, Heavy metals, Nitrogen, and Phosphorous accumulation. J Environ Qual 31:1522–1527.
Kulling DF, Stadelmann, Herter U (2001) Sewage sludge – Fertilizer or waste? UKWIR Conference Brussels.
Kuperman RG, Carreiro MM (1997) Soil heavy metal concentrations, microbial biomass and enzyme activities in a contaminated grassland ecosystem. Soil Biol Biochem 29:179–190.
Laturnus F, Von AK, Gron C (2007) Organic contaminants from sewage sludge applied to agricultural soils - False alarm regarding possible problems for food safety? Environ Sci Pollut Res 14:53–60.
Leita L, Noblil M, Muhibachova G, Mondini C, Marchiol L, Zerbi G (1995) Bioavailability and effects of heavy metals on soil microbial biomass survival during laboratory incubation. Biol Fertil Soils 19:103 –106.
Lepp NW (1981) Effect of heavy metal pollution on plants. In: Metals in the environment, vol 2. Applied Science London.

Lewis DL, Gattie D K (2002) Pathogen Risks From Applying Sewage Sludge to Land. Environ Sci Tech 287–293.
Liu X, Zhang S, Wu W, Liu H (2007) Metal sorption on soils as affected by the dissolved organic matter in sewage sludge application. J Hazard Mate 149:399–407.
Logan TJ, Harrison BJ (1995) Physical characteristics of alkaline stabilized sewage sludge (N-vitro soil) and their effects on soil properties. J Environ Qual 24:153–64.
Lopez-Mosquera ME, Moiron C, Carral E (2000) Use of dairy industry sludge as fertilizer for grassland in northwest Spain: heavy metal levels in the soil and plants. Resour Conserv Recy 30:95–109.
Magdoff FR, Amadon JF (1980) Nitrogen availability from sewage sludge. J Environ Qual 9: 451–455.
Mahdavi M, Jafari J (2010) Environmental Risks due to Application of Sewage Sludge in Farmlands. Ozean JAppl Sci 3. ISSN 1943–2429.
Maiti PS, Sah KD, Gupta SK, Banerjee SK (1992) Evaluation of sewage sludge as a source of irrigation and manure. J Indian Soc Soil Sci 40:168–172.
Martinez F, Cuevas G, Walter T, Iglesias I (2002) Urban organic wastes effects on soil chemical properties in degraded semiarid ecosystem.17th WCSS, Symposium No20 Thailand pp1–9.
McBride MB (1995) Toxic metal accumulation from agricultural use of sludge: Are USEPA regulations prospective? J Environ Qual 24:5–18.
McBride MB, Richards BK, Steenhuis T,. Russo JJ, Sauve S (1997) Mobility and solubility of toxic metals and nutrients in soil fifteen years after sludge application. Soil Science 162: 487–500.
McBride MB, Richards BK, Steenhuis T, Spiers G (1999) Long-term leaching of trace elements in a heavily sludge-amended silty clay loam soil. Soil Science164:613–623.
McCarthy JF, Zachara JM (1989) subsurface transport of contaminants. Environ Sci Technol 23: 496–502.
Mc Grath S, Postma L, McCormack RJ, Dowdall C (2000) Analysis of Irish sewage sludges: suitability of sludge for use in agriculture. Irish J Ag Food Res 39:73–78.
Mc Grath SP, Chang AC, Page AL, Witter E (1994) Land application of sewage sludge: scientific perspectives of heavy metal loading limits in Europe and the United States. Envir Rev 2:108–118.
Metcalf, Eddy (2003) Wastewater engineering: Treatment, disposal and reuse. 4th edition. New York: McGraw-Hill Publishing Company Ltd.
Moreno JL, Garcia C, Hernandez T, Ayuso M (1997) Application of composted sewage sludges contaminated with heavy metals to an agricultural soil: Effect on lettuce growth. Soil Sci Plant Nutr 4:565–573.
Morera MT, Echeverria J, Garrido J (2002) Bioavailability of heavy metals in soils amended with sewage sludge. Can J Soil Sci 82:433–438.
Naidu R, Bolan NS, Kookana R S, Tiller K G (1994) Ionic-strength and pH effects on the sorption of cadmium and the surface charge of soils. Eur J Soil Sci 45:419–429.
Nandakumar K, Ramamurthy S, Rajarajan, A Savarimuthu E (1998) Suitability of Dindigul town's sewage sludge for field application: Nutritional perspective Poll Res 17:61–63.
Nielson GH, Hogue EJ, Nielson D, Zebarth BJ (1998) Evaluation of organic wastes as soil amendments for cultivation of carrot and chard on irrigated sandy soils. Can J Soil Sci 78:217–225.
Ojeda G, Alcaniz JM, Ortiz O (2003) Runoff and losses by erosion in soils amended with sewage sludge. Land Degrad Dev14:563–573.
Pahren H, Jakubowski W (1980) USEPA 600/9-80-028. USEPA Cincinnati OH.
Parat C, Chaussod R, Leveque J, Andreux F (2005) Long-term effects of metal-containing farmyard manure and sewage sludge on soil organic matter in a fluvisol. Soil Biol Biochem 37: 673–679.
Parkpain P, Sirisukhodom S, Carbonell-Barrachina AA (1998) Heavy metals and nutrients chemistry in sewage sludge amended Thai soils. J Environ Sci Health 33:573–597.
Pascual JA, García G, Hernández T, Moreno JL, Ros M (2000) Soil microbial activity as a biomarker of degradation and remediation processes. Soil Biol Biochem32:1877–1883.
Paul EA, Clark FE (1996) Soil Microbiology and Biochemistry. San Diego, CA, Academic Press.

Powelson DK, Simpson JR, Gerba CP (1991) Effects of organic matter on virus transport in unsaturated flow. Appl Environ Microbiol 57:2192–2196.
Powlson DS (1994) Quantification of nutrient cycles using long-term experiments. pp 97–115. In R.A. Leigh and A.E. Johnston (eds) Long-term experiments in agricultural and ecological sciences. CAB Int Wallingford, Oxon UK.
Ramulu US Sree (2002) Reuse of municipal sewage and sludge in agriculture. Scientific Publishers, Jodhpur India.
Rose J, Dickson L, Farrah S, Carnahan R (1996) Removal of pathogenic and indicator microorganisms by a full-scale water reclamation facility. Water Res30:2785–2797.
Russ CF, Yanko WA (1981) Factors affecting Salmonellae repopulation in composted sludges. Appl Environ Microbiol 41:597–602.
Sanchez Monedero MA, Mondini C, Nobili M De, Leita L, Roig A (2004) Land applications of biosolids. Soil response to different stabilization degree or treated organic matter. Waste Manage 24:325–332.
Santos I, Bettiol W (2003) Effect of sewage sludge on the rot and seedling damping-off of bean plants caused by *Sclerotium rolfsii*, Crop Prot 22:1093–1097.
Sidhu J (2000) Microbial Health Hazards Associated with the Land Application of Biosolids, Murdoch University, Perth, Western Australia.
Sidhu J, Gibbs RA, Ho GE, Unkovich I (2001) The role of indigenous microorganisms in suppression of Salmonella regrowth in composted biosolids. Water Res 35:913–920.
Sigua GC (2005) Current and future outlook of dredged and sewage sludge materials in agriculture and environment, J Soils Sediments 5:50–52.
Silva JD, Leal TT, Araújo AS, Araujo RM, Gomes RL, Melo WJ, Singh RP (2010). Effect of different tannery sludge compost amendment rates on growth, biomass accumulation and yield responses of Capsicum plants. Waste Manage 30:1976–1980.
Singh RP, Agrawal M (2007) Effects of sewage sludge amendment on heavy metal accumulation and consequent responses of *Beta vulgaris* plants. Chemosphere 67:2229–2240.
Singh RP, Agrawal M (2009) Use of sewage sludge as fertilizer supplement for *Abelmoschus esculentus* plants: Physiological, biochemical and growth responses. Int J Environ Waste Manage 3:91–106.
Singh RP, Agrawal M (2010a) Biochemical and physiological responses of Rice *Oryza sativa* L grown on different sewage sludge amendments rates. Bull Environ Contam Toxicol 23: 606–612.
Singh RP, Agrawal M (2010b) Effect of different sewage sludge applications on growth and yield of *Vigna radiata* L. field crop: Metal uptake by plant. Ecol Eng 36:969–972.
Singh RP, Agrawal M (2010c) Variations in heavy metal accumulation, growth and yield of rice plants grown at different sewage sludge amendment rates. Ecotoxicol Environ Saf 73: 632–641.
Singh RP, Agrawal M (2008) Potential benefits and risks of land application of sewage sludge. Waste Manage 28:347–358.
Skujins J (1978) Soil enzymology and fertility index- a fallacy? History of abiotic soil enzyme research. In: Burns RG (eds) Soil Enzymes Academic Press London pp 1–49.
Sommers LE (1977) Chemical composition of sewage sludges and analysis of their potential use as fertilizers. J Environ Qual 6:225–232.
Sommers LE, Nelson DW, Yost KJ (1976) Variable nature of chemical composition of sewage sludges. J Environ Qual 5:303–306.
Soon YK (1981) Solubility and sorption of Cadmium in soils amended with sewage sludge. J Soil Sci 32:85–95.
Suhadolc M, Schroll R, Gattinger A, Schloter M, Munch JC, Lestan D (2004) Effects of modified Pb, Znand Cd availability on the microbial communities and on the degradation of isoproturon in a heavy metal contaminated soil. Soil Biol Biochem 36:1943–1954.
Suhadolc M, Schroll R, Hagn A, Dörfler U, Schloter M, Lobnik F (2010) Single application of sewage sludge Impact on the quality of an alluvial agricultural soil. Chemosphere doi:10.1016/j.chemosphere.2010.08.024.

Tabatabai MA (1994) Soil enzymes. In: Weaver RW, Angle JS, Bottomley PS (eds) Methods of soil analysis, part 2. Microbiological and biochemical properties. SSSA Book Series No. 5. Soil Sci Soc Amer Madison, WI, pp 775–833.

Tsadilas CD, Matsi T, Barbayiannis N, Dimoyiannis D (1995) Influence of sewage sludge application on soil properties and on the distribution and availability of heavy metal fractions. Comm Soil Sci Plant Anal 26:2603–2619.

USEPA (1994) Guide to Septage Treatment and Disposal. EPA/625/R-94/002. Office of Research and Development. Washington DC.

Udom B E, Mbagwu JSC, Adesodun JK, Agbim NN (2004) Distributions of zinc, copper, cadmium and lead in a tropical ultisol after long term disposal of sewage sludge. Environ Int 30:467–470.

USEPA (1999a) Control of pathogens and vector attraction in sewage sludge, United States Environmental Protection Agency (USEPA), Washington DC.

USEPA (1999b) Phytoremediation resource guide. Washington: U.S. Environmental Protection Agency; EPA 542-B-99-003.

Vaidya C (2009) Urban issues, reforms and way forward India. Working Paper No.4/2009-DEA. Department of Economic Affairs, Ministry of Finance, Government of India.

Viera RF, de Souza Silva C M M (2003) Soil amendment with sewage sludge and its impact on soil microflora. Braz J Microbiol 34:56–58.

Wang P, Qu E, Z Li, Shuman LM (1997) Fractions and availability of nickel in Loessial soil amended with sewage or sewage sludge. J Environ Qual 26:795–801.

Zhidong Li, Wenjing Li (2009) Technological Parameters of Exceed Sludge Anaerobic Digestion in Industrial Wastewater Treatment Plant. The Electronic J Geotech Eng Vol. 14.

Accumulation of Heavy Metals in Selected Medicinal Plants

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1 Introduction

Heavy metals (HMs) are natural components of the Earth's crust and are usually present in all environmental matrices. However, the concentration of several HMs has increased several fold in some ecosystems as a result of anthropogenic activities. Heavy environmental metal contamination has continued to gain global attention, mainly because of the toxicological risks posed by such metals to human health (Ayodeji and Olorunsola 2011). Although metallic elements are often essential for living organisms, they become toxic when present at high concentrations (Elekes et al. 2010). The rapid increase in human population, coupled with haphazard industrialization and technological advancement, has caused many serious environmental problems around the world; among the causes of such problems is the production and release of toxic metals. In the past few decades, the concentration of heavy metals in

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soil and surface waters has increased (Nriagu and Pacyna 1988; Larison et al. 2000) and now constitutes a potential threat to terrestrial and aquatic biota (Ives and Cardinale 2004; Nasim and Dhir 2010) and to humans by entering the food chain (Hsu et al. 2006; Meena et al. 2008). Because of the widespread presence of heavy metals in the environment, their residues also reach and are assimilated into medicinal plants.

Any nonbiologically degradable metal or metalloid that causes an environmental problem should be considered to be a "heavy metal" (Herrera-Estrella and Guevara-Garcia 2009). Fifty three elements now fall into the category that can be properly be referred to as heavy metals; such elements are defined as the group of elements whose densities are greater than 5 $g\,cm^{-3}$ (Sarma 2011). The responses that plants display to a HM-contaminated environment for purposes of adaptation are determined by many physiological, molecular, genetic, and ecological traits. But in general, exposure to very high HM concentrations affects both the growth and metabolism of plants (Dhir et al. 2009).

Traditional healers often prescribe mixtures of medicinal plants in raw form for diseases ranging from common cold to malaria, arthritis, ulcers, hepatitis, and diabetes among others (Obiajunwa et al. 2002; Sarma and Sarma 2008; Sarma et al. 2008). The use of medicinal plants/herbal products as the first choice in self-treatment continues to expand rapidly across the developing world, viz., India, China, and South Africa, to prevent imminent development of certain diseases. In primary healthcare, about 70–80% of the world's population rely on unconventional medicine, mainly of herbal origin (WHO 2002), and in some parts of the world, herbal medicines are the only options for primary health care for poor people. In Ethiopia, more than 85% of the population depends on herbs for primary health care (Meena et al. 2010). Medicinal plants are particularly important in developing countries because such plants are also dietary components and are essential for health (Maiga et al. 2005; Cantarelli et al. 2010); this, therefore, accounts for the increasing popularity of phytotherapy (Gjorgieva et al. 2010). The average annual volume of medicinal and aromatic plants that are utilized in EU countries has increased by 21% since 1992, meaning that more than 100,000 ton of herbal drugs, worth US$330 million, are used in traditional or processed forms (Bernath 2002). Most medicinal plants are rich in minerals and metals and are usually consumed in low doses as nonprescription herbal drugs for debility prevention (Hay 1984; Obiajunwa et al. 2002). Despite widespread use, however, the adverse effects of long-term ingestion of high doses of minerals and metal supplements are still not well undocumented (Ivey and Elmen 1986).

In India, about 2,000 drugs of plant origin are used. The distribution of medicinal plants is now under great pressure in India because excessive amounts of them are collected from wild habitats and are exploited for use in medicine. Such overcollection has endangered 20–25% of existing plant species in India (Laloo et al. 2006). Medicinal plants are normally procured indiscriminately from noncultivated wild habitats by untrained and uneducated people, and pushed to the market or raw drug suppliers of the pharmaceutical industry without any analysis of the plants for metal content. Such premarket analysis is pertinent to ensure that levels of different toxic heavy metals do not exist in polyherbal medical formulations (Meena et al. 2010).

Lead, in particular, continues to be a significant public health problem in developing Asian countries, and the threat is not only to humans but also to various species of terrestrial organisms (Hsu et al. 2006). Pb is toxic and has no known biological function in humans. Numerous studies have disclosed that traditional Indian and Chinese herbal preparations do contain high levels of lead and other toxic heavy metals. For example, 64% of herbal samples collected in India displayed substantial amounts of lead and other heavy metal residues (Ernst 2002). In addition to Pb, Cd, and Hg are also known to be toxic to humans, even at very low concentrations, and these metals are taken up by some medicinal plants from the environment (Chen 1992). Although herbal products are preferred by consumers for their "natural origin," and are believed to be devoid of harmful effects, some medicinal plants accumulate heavy metals from polluted sources and pose a toxic threat to human consumption. Although herbal medicines may be as efficient as synthetic ones, they may be less safe because of the presence of unacceptably high levels of certain heavy metals (Blicharska et al. 2010).

The toxicity to biota of heavy metals has been of interest to scientists for many years. In recent decades, to assure the quality of herbal medicines, phytopharmaceuticals and herbal medicines have been extensively analyzed by laboratories around the world for their metal content (Narendhiraknnan et al. 2005; Garg et al. 2007). Moreover, the World Health Organization (WHO) and the US Food and Drug Administration (FDA) have standardized safe limits for the occurrence of certain metals (e.g., As, Hg, Pb, and Cd) in herbal drugs. However, the WHO has not yet decided what the permissible limits in medicinal plants are for all metals, because many of these metals are also essential dietary micronutrients for humans.

An essential element is one that is required for maintenance of life, whose deficient intake consistently results in a functional impairment from some optimal to a suboptimal level. Supplementation of an essential deficient element to proper physiological levels prevents or cures the impairment (Mertz 1981). Certain metals are among micronutrients that are important for normal functioning of vital organs. Metals are critical components of many enzymes, e.g., zinc is a cofactor for more than 100 metalloenzymes (Kosalec et al. 2009), and are essential for proper functioning of the biochemical processes in which they participate (Lozak et al. 2002). Nevertheless, at sufficient concentrations, several of these essential metals are potentially toxic (Donkin and Ohlson 2000; Obi et al. 2006).

Medicinal plants are mainly sourced and harvested from wild habitats, after which they are delivered to their market, without many questions being asked about their origins, botanical identity, purity, safety, and efficacy. The main reason that purchasers lack inquisitiveness is the cost factor. Although harvesting from nature ensures sufficient volumes and low cost of these plants, if the buyer wants to verify the background and quality of them, the cost increases. At the current time, medicinal plants growing in the wild constitutes an important portion of herbal plants that are traded in Europe (Salgueiro et al. 2010). Because of the foregoing points, a fundamental issue concerning the medicinal plant industry has to be the quality of these plants. The safety and benefit of plant products are directly related to the quality of the raw materials from which they are derived (Salgueiro et al. 2010).

Several regulations have already been established globally for medicinal plants and related marketed herbal products; among the regulating entities are the following: the US Pharmacopoeia (USP), Italian Pharmacopoeia (FUI), and European Pharmacopoeia (Ph. Eur.). Moreover, there are legal frameworks at national or regional levels that are designed to regulate the quality of herbal products. However, the existing medicinal plant regulatory guidelines are quite complicated (Kosalec et al. 2009), which at times render them more difficult to follow. There are also differences in standards and regulations among countries, which may increase confusion, and cause a situation in which health risks to consumers increases.

The literature that addresses quantification of heavy metals in medicinal plants is extensive and, as we have observed in preparing this review, when assembled, contributes to a clearer understanding of the pattern for how metals accumulate in such plants. In this context, our goals in this review are to evaluate the limits set by governmental agencies for heavy metal safety in selected medicinal plants and to compare these with the actual levels found in these plants. We also address some of the uses and effectiveness to which medicinal plants are put in health care, and mention some hazards associated with the use of medicinal plants.

2 Medicinal Plants in Health Care: Use and Effectiveness

Humans use many wild plant species for nutrition, for enhancing food security, for medicines, and for sustainable development and livelihood management. In addition, the world's forests provide humans with several life-supporting commodities; it is estimated that 350 million people worldwide live within or adjacent to dense forests and depend on them for subsistence (Arnold 2001). In sub-Saharan Africa (SSA) and Asia, the majority of people living in rural communities survive on less than US $1 a day (World Bank 2001, 2004; FAO 2005; CIFOR 2002), and these people largely sustain their livelihoods from forests. Most medicinal plants used in traditional health-care systems contribute significantly to the caloric intake and dietary nutrition of people who consume them, and such intake enhances human health and cures various ailments. Aboriginal and tribal peoples are remnants of primitive societies, and many such peoples continue to live close to nature, where they have acquired unique knowledge concerning the sustainable use of wild medicinal plants. This knowledge has interestingly often spread to modern society, along with the belief that such natural drugs are better and safer than synthetic ones.

Despite being based culturally on different theoretical models, medicinal plants were used in all traditional medicinal systems, viz., Ayurveda, Chinese, Unani, Tibetan, Amazonian, and African, which integrated phytotherapy into their doctrines (WHO 2007). Herbal medicines were and are often preferred in health care over more modern chemicals, despite the fact that they may contain contaminating chemicals obtained directly from contact with polluted air, water, and soil (Shamsa et al. 2009). Medicinal plants are, nonetheless, used globally in many aspects of

health care, although perhaps preferentially in developing countries. Medicinal plants are in demand both because of their natural origins and lower costs compared to synthetic drugs. In India, where 74% of the population resides in its seven million villages, the use of medicinal plants in health care may also be welcomed as a strategy for assisting in the development of the hitherto underdeveloped countryside.

Currently, nutraceuticals from plants have gained popularity with consumers, who are increasingly careful in choosing components of their diet; such people are interested in incorporating high nutrient levels into their standard diet, preferably sourced naturally from plants (Bhat and Sridhar 2008; Bhat et al. 2010). There are at least 50 elements that are vital for the well-being of humans (Tolonen 1990). Dietary elements required in amounts greater than 100 mg/day are called "minerals," and those that are required in amounts less than 100 mg/day are called "trace elements." Trace elements are necessary for human health and include the following: iron, copper, manganese, zinc, selenium, chromium, etc. (Hendler and Sheldon 1990). Minerals include calcium, magnesium, phosphorus, sodium, potassium, sulfur, and chlorine. It is well established that a majority of trace elements act as key components of essential enzyme systems or other proteins, e.g., the hemoprotein or hemoglobin, which perform vital biochemical functions (Obiajunwa et al. 2002). Many medicinal plants contain elements of vital importance, which are needed for growth and development, for prevention and healing of diseases, and the plants that provide such benefit have long been heavily used in Asia and Africa.

Both deficiencies and excesses of dietary metals may result in several human disorders. Some toxic metals are capable of damaging vital organs even at extremely low concentrations, e.g., Pb causes both acute and chronic poisoning and also poses adverse effects on the kidney, and liver, and on the vascular and immune systems (Heyes 1997). Heavy metals are the main cause of excessive free radical activity, which can also cause damage to healthy tissues of the body and can deplete the body's immune system (Cranton and Frackelton 1998).

The uptake of heavy metals by plants may be duration-dependent, and once absorbed by plants, they may accumulate in certain plant parts (Khan et al. 2007). In some plant species, prolonged exposure to metals may result in levels that, when consumed, are hazardous to humans. The international agency for research on cancer (IARC) identified the following metals as being potentially carcinogenic to humans: arsenic, antimony, beryllium, cadmium, chromium, cobalt, lead, nickel, and vanadium. Moreover, some of these toxic heavy metals can cause DNA damage; hence, the carcinogenic effects they produce in animals and humans may result from mutagenic activity. It is because of the potential intake by humans of potentially toxic amounts of heavy metals that researchers have emphasized the importance of monitoring for the presence of such carcinogenic metals in medicinal plants, to hopefully prevent excessive human exposures (Liang et al. 2004; Arceusz et al. 2010). Moreover, medicinal plants are used in folk medicine, and knowledge of the metal content, is important for this reason (Chuparina and Aisueva 2011).

3 Heavy Metal Accumulation in Selected Medicinal Plants: Cause and Threat

Many papers have been published that address the analysis of heavy metals in medicinal plants; the results presented in these papers have improved insights into what the heavy metal accumulation levels of some medicinal plants are, or may be (Ajasa et al. 2004; Koe and Sari 2009; Sharma et al. 2009; Sheded et al. 2006; Wong et al. 1993). In Table 1, we have summarized data from the literature on the metal concentrations found in 88 medicinal plants. These data clearly illustrate that zinc is the metal that is most commonly found in medicinal plants, and is present in all species tested. These data also reveal that metals vital for human health are present in many plant species at variable concentrations. For example, Fe and Cu were found in 87 of the surveyed plant species, Cl in 23 species, whereas Se and Au were found in 21 and 9 species, respectively. However, many medicinal herbs accumulate heavy metals (e.g., As, Cd, Pb, and Hg), which are variably hazardous to humans, in ways that are dependent on their oxidation states and their concentrations (Lekouch et al. 2001).

Certain metals (e.g., Cr, Mn, Zn, and Cu) usually appear in green plants at low concentrations, and the health of organisms consuming such plants is unaffected. However, under certain environmental conditions, the concentration of metals may be higher. For example, copper plant levels increase when the soil pH is low and when organic fertilizers are used (Elekes et al. 2010). Similarly, in wet soils having an acidic pH, half of the zinc present is bound to organic matter (Domany et al. 1996), binding increases and zinc levels can reach 5,000 mg/kg (Elekes et al. 2010). Some plant species that absorb high metal levels have developed the ability to detoxify them by forming metal-binding peptides called phytochelatin (PCs). Such plants retain great capacity to absorb metals from the soil and transfer these metals to plant-consuming biomass. Heavy metal ions present in plants are absorbed by roots, taken to aerial plant parts and are bioaccumulated, along with any essential metals that are present, such as Mn, Fe, Cu, Zn, and Se (Maiga et al. 2005). This is an active physiological process, which requires energy (Fox and Guerinot 1998). Depending on the plant species involved, plants base their tolerance to heavy metals on two basic strategies: exclusion and accumulation (De Vas et al. 1991). The accumulation strategy involves physiological processes that require the cells to maintain the intracellular heavy metal ions, but in a nontoxic form (Cobbet 2000). Exclusion occurs when any stored heavy metal ions or complexes are later removed by leaf fall (Ernst et al. 1992). Accumulation of heavy metals in plant tissues is affected by the characteristics of soil and atmosphere, and the uptake ability of plants (Bin et al. 2001). As Table 1 shows, medicinal plants do accumulate heavy metals in considerable quantities. In some marketed medicinal plants, the concentrations increase to hazardous levels. Despite the presence of high metal levels, medicinal plants are still used in phytopharmaceuticals. Therefore, one may assume that some proportion of medicinal plants that are grown in metal contaminated soils are fated to suffer phytotoxicity, and HM residues from them may reach the food chain and pose a threat to human health (Oliver 1997; Maharia et al. 2010).

4 Safe Limits vs. Actual Levels of Heavy Metals in Medicinal Plants

Among the 88 medicinal species tested for metal contamination, the number of species in which safe levels were exceeded was as follows: Pb in 21 species, Cd in 44 species, and Hg in 10 species (Table 2). This is extremely disquieting and explains why metal contamination of marketed medicinal plants is often criticized. The chronic accumulation of heavy metals in certain vital human organs from prolonged ingestion of these plants has been well established (Sharma et al. 2009; WHO 2005). Hence, the importance to consumer protection of having good quality control practices for screening of herbal medicines, particularly for heavy metals, is underscored.

Although permissible levels of heavy metal contaminants in herbal medicine are not yet standardized, the European Pharmacopoeia (Ph. Eur.) has drafted limits for Cd, Pb, and Hg (Table 3). Furthermore, the European Commission established limits in March 2001 for lead, cadmium, and mercury in food supplements (Commission Regulation 2001). The WHO and the Food and Agriculture Organization (FAO) have also jointly proposed acceptable levels of toxic substances that can be ingested on a weekly basis – the Provisional Tolerable Weekly Intake (PTWI) for As, Cd, Hg, and Pb (International Program on Chemical Safety 2009). In addition, the United States has standardized recommended daily dietary allowance (RDA) for essential dietary metals, but not for toxic ones.

However, there are few established safe limits for the content of heavy metals in, or permissible limits for minerals in medicinal plants (Wang et al. 1985). This dearth invariably hamstrings development of medicinal plant research. The governmental agencies responsible for establishing such safe limits for essential metallic minerals or for metal content in medicinal plants must address appropriate standard-setting with the urgency it deserves. WHO, which is one of the monitoring bodies, should intervene to help identify what limits are necessary and to establish and standardize such limits.

5 Hazardous Medicinal Plants

Having heavy metal analytical results available on medicinal plants is the critical step in knowing if such plants have potentially toxic levels of metals that could be dangerous to living organisms. Moreover, having data on hyperaccumulator plants is even more critical. Such plants accumulate high concentrations of metallic elements and pose special risks to consumers, because they can absorb 50–100 times the amount of metals than do normal plants (Chaney et al. 1997). The phenomenon of hyperaccumulation is intensified if the environment has high concentrations of heavy metals. To date, about 500 plant species are recognized to be hyperaccumulators; this number represents approximately 0.2% of all angiosperm species

Table 1 Concentrations of metals (μg/g) present in different medicinal plant parts

Species	Dry Weight	Al	As	Au	Br	Cd	Cl	Co	Cr
Acalypha wilkesiana	Leaf	***	***	***	10.6	***	***	***	34.6
Achyranthes aspera	Whole plant	***	***	***	***	0.59	***	5.23	1.48
Acorus calamus	Leaf and root	1,360	***	0.00018	9.78	***	1,930	0.19	2.01
Aegle marmelos	Leaf	***	***	***	***	***	***	***	1.73
Aframomum melegueta	Seed	***	***	***	43.4	***	***	***	***
Alchornea cordifolia	Leaf	***	***	***	4.5	***	***	***	23
Aloe vera	Leaf	13.27	***	***	***	0.051	***	0.116	***
Alternanthera pungens	Whole plant	***	***	***	***	1.45	***	3.41	17.74
Amacylus pyrethrom	Root	***	***	***	***	6.598	0.71	7.298	2.499
Andrographis paniculata	Fruit	2,470	***	***	8.45	***	2,460	0.16	1.11
Anethum sowa	Seed	***	***	***	***	3.896	***	8.634	3.068
Artemisia nilagirica	Leaf	***	0.3	***	5.7	***	***	***	1.3
Azadirachta indica	Leaf	960	***	0.00384	28.8	***	2,010	0.12	1.47
Bergemia liquilata	Root	***	***	***	***	704.39	***	4.985	1.389
Boerhavia diffusa	Leaf	***	***	***	***	37.5	***	***	***
Brassica campestris	Whole plant	***	***	***	***	1.2	***	7.55	8.19
Calotropis procera	Leaf	***	***	***	22.4	***	***	***	198.6
Cannabis sativa	Leaf	***	***	***	***	1.66	***	4.79	29.49
Carcum carvi	Seed	***	***	***	***	5.481	370	10.662	3.587
Carica papaya	Leaf	***	***	***	***	0.899	***	1.698	4.595
Carum roxburghianum	Seed	***	***	***	***	***	1,240	3.799	***
Cassia alata	Leaf	***	***	***	5.6	***	***	***	***

Cu	Fe	Hg	Mn	Ni	Pb	Se	V	Zn	Sample location	Reference
146.1	760.1	***	44.4	203.6	***	17.9	27.1	75.3	Nigeria	Obiajunwa et al. (2002)
7.06	455.9	***	105.6	5.9	9.28	***	***	20.91	Pakistan	Jabeen et al. (2010)
6.4	80.2	***	121	***	***	***	0.66	37.9	India	Garg et al. (2007)
0.38	2.67	***	***	0.81	***	***	0.24	0.14	India	Narendhiraknnan et al. (2005)
247.8	333.2	***	232.3	245.9	***	41.3	42.1	120	Nigeria	Obiajunwa et al. (2002)
124	161.1	***	274.1	130.1	***	8.5	339.9	71.3	Nigeria	Obiajunwa et al. (2002)
1.733	18.59	***	0.976	0.291	0.621	***	7.545	>200	India	Rajendran et al. (2007)
9.11	739.5	***	40.5	7.97	9.89	***	***	37.86	Pakistan	Jabeen et al. (2010)
13.595	1259.6	99.9	36.62	42.79	5.99	***	***	39.49	India	Rajurkar and Damame (1997)
11.4	250	***	51.3	***	***	***	1.44	30.9	India	Garg et al. (2007)
22.064	1064.5	***	52.2	28.66	12.38	***	***	32.08	India	Rajurkar and Damame (1997)
23.2	820.4	***	123	5.6	***	0.3	12.3	33.7	India	Singh et al. (2010)
6.49	256	***	46.4	***	***	***	1.19	34.8	India	Garg et al. (2007)
11.707	334	505.9	26.9	23.02	9.21	***	***	36.81	India	Rajurkar and Damame (1997)
10.5	358.5	***	22.5	***	***	***	***	43.5	Ghana	Annan et al. (2010)
10.78	1,890	***	87.01	6.64	8.78	***	***	37.56	Pakistan	Jabeen et al. (2010)
137.8	1,872	***	231.5	235.2	***	15.9	***	71.7	Nigeria	Obiajunwa et al. (2002)
9.6	387.6	***	54.19	15.8	10.57	***	***	29.45	Pakistan	Jabeen et al. (2010)
21.723	1135.9	***	83.77	51.11	22.95	***	***	57.09	India	Rajurkar and Damame (1997)
10.389	2,254	49.9	20.99	25.47	0.09	***	***	7695.4	India	Rajurkar and Damame (1997)
7.099	416.5	***	15.36	13.09	***	***	***	82.99	India	Rajurkar and Damame (1997)
97.8	3,924	***	178.9	92.8	***	8.6	116.5	46.9	Nigeria	Obiajunwa et al. (2002)

(continued)

Table 1 (continued)

Species	Dry Weight	Al	As	Au	Br	Cd	Cl	Co	Cr
Cassia rhombifolia	Fruit	0.2	***	0.48	4.45	***	1.41	0.55	1.47
Cassia siamea	Leaf	***	***	***	***	30	***	***	***
Catharanthus roseus	Leaf	***	***	***	6	***	***	***	24.6
Celastrus paniculata	Seed	***	***	***	***	1.399	***	9.597	5.898
Chromolaena odorata	Leaf	***	***	***	21.5	***	***	***	***
Cinnamomum zeylanicum	Leaf	***	***	***	***	42.5	***	***	***
Citrus aurantifolia	Leaf	***	***	***	2.3	***	***	***	***
Clausena anisata	Leaf	***	***	***	***	28.5	***	***	***
Clematis gouriana	Root	***	***	***	***	864.91	***	6.462	4.971
Convolvulus arvensis	Whole plant	***	***	***	***	1.23	***	4.33	1.2
Cyclosorus afer	Leaf	***	***	***	8.6	***	***	***	***
Datura metel	Leaf and fruit	***	***	***	11.6	***	***	***	15.4
Desmodium adscendens	Leaf	***	***	***	***	50	***	***	***
Elsholtzia communis	Inflore-scence	***	2.1	***	8.1	***	***	***	32.6
Embelia ribes	Fruit	1,240	***	0.00026	15	***	5,940	0.23	3.02
Emblica officinalis	Fruit	***	***	***	6.7	***	***	***	***
Eugenia uniflora	Leaf	***	***	***	4.5	***	***	***	21.3
Euphorbia hirta	Aerial parts	***	***	***	18.4	***	***	***	***
Ficus exasperate	Leaf	***	***	***	18	***	***	***	***
Garcinia kola	Seed	***	***	***	26.2	***	***	***	***
Garcinia pedunculata	Stem	***	***	***	***	3.288	***	8.203	1.458
Gentiana kurroa	Leaf	***	***	***	***	6.295	***	13.289	3.497
Gmelina arborea	Root	***	***	***	***	1.264	1,270	3.922	5.604

Cu	Fe	Hg	Mn	Ni	Pb	Se	V	Zn	Sample location	Reference
2.63	697	***	6.44	***	***	***	***	38.9	India	Garg et al. (2007)
11.5	156	***	4	16.5	***	***	***	47	Ghana	Annan et al. (2010)
3.7	424.9	***	183.7	6.8	***	***	***	54.6	India	Singh et al. (2010)
11.296	13,295	279.9	20.76	30.29	11.99	***	***	4678.13	India	Rajurkar and Damame (1997)
228.3	261.2	***	187.7	201.2	***	8	***	95	Nigeria	Obiajunwa et al. (2002)
22.5	***	***	233.5	***	***	***	***	46.5	Ghana	Annan et al. (2010)
19.3	125	***	12.13	24.6	***	1.6	170.5	8.7	Nigeria	Obiajunwa et al. (2002)
8	639.5	***	64.5	22	***	***	***	495	Ghana	Annan et al. (2010)
12.725	208.2	328.1	56.37	16.5	17.89	***	***	81.02	India	Rajurkar and Damame (1997)
8.93	391.9	***	77.35	2.6	3.15	***	***	17.38	Pakistan	Jabeen et al. (2010)
123	625.2	***	113.4	121.5	***	7.8	37.2	76.2	Nigeria	Obiajunwa et al. (2002)
***	674.2	***	64.1	99.2	***	7.9	320	40.2	Nigeria	Obiajunwa et al. (2002)
63.5	247	***	35	***	***	***	***	164.5	Ghana	Annan et al. (2010)
30.1	751.3	***	229	7.6	***	0.5	17.8	62.8	India	Singh et al. (2010)
22.7	78.1	***	50.2	***	***	***	3.55	37.7	India	Garg et al. (2007)
5.9	311.1	***	111	7	***	***	***	23.3	India	Singh et al. (2010)
172.2	308.6	***	49.5	231.8	***	8.7	23.6	97.5	Nigeria	Obiajunwa et al. (2002)
234.8	535.7	***	52	241.1	***	16.8	***	191.1	Nigeria	Obiajunwa et al. (2002)
99.3	187	***	126.2	107.6	***	6.7	32.7	51.5	Nigeria	Obiajunwa et al. (2002)
188.6	228.7	***	53.5	171.2	***	23.6	***	105.3	Nigeria	Obiajunwa et al. (2002)
6.439	120.4	***	35.08	23.76	8.69	***	***	40.1	India	Rajurkar and Damame (1997)
13.989	719.4	***	302.3	56.56	16.99	***	***	40.07	India	Rajurkar and Damame (1997)
5.468	103.3	***	35.29	110.1	***	***	***	42.74	India	Rajurkar and Damame (1997)

(continued)

Table 1 (continued)

Species	Dry Weight	Al	As	Au	Br	Cd	Cl	Co	Cr
Gymnema sylvestre	Leaf	***	***	***	***	53	***	***	***
Heliotropium indicum	Leaf	***	***	***	***	28.5	***	***	***
Hemidesmus indicus	Root	0.25	***	0.38	1.73	***	2.4	0.19	2
Holarrhena antidysenterica	Leaf	1.04	***	***	23.9	***	2.34	0.22	1.3
Hordeum vulgare	Seed	***	***	***	***	1.16	***	11.26	6.21
Jatropha gossypyfolia	Leaf	***	***	***	***	35.5	***	***	***
Justicia adhatoda	Whole plant	***	***	***	***	0.99	***	6.5	5.3
Laportea aestuans	Aerial parts	***	***	***	11.9	***	***	***	***
Mangifera indica	Leaf	***	***	***	36.1	***	***	***	***
Mentha piperitae	Leaf	***	***	***	***	***	***	***	3.6
Momordica charantia	Aerial parts	***	***	***	4.2	***	***	***	***
Murraya koenigii	Leaf	***	***	***	***	***	***	***	1.5
Myrstica fragrans	Fruit	30	***	0.00136	0.51	***	230	0.19	1.91
Nardostachys jatamansi	Root	1,700	***	***	7.28	***	1,190	1.26	8.19
Nelumbo nucifera	Flower	***	***	***	***	0.198	***	4.165	1.587
Ocimum gratissimum	Leaf	***	***	***	13.9	***	***	***	***
Ocimum tenuiflorum	Seed	***	***	***	***	1.856	***	22.944	9.669
Parthenium hysterophorus	Whole plant	***	***	***	***	1.19	***	4.93	6.07
Pergularia daemia	Leaf	***	***	***	***	26.5	***	***	***
Phyllanthus amarus	Leaf	***	***	***	***	27.5	***	***	***
Psoralea corylifolia	Seed	1,340	***	***	5.24	***	1,020	0.86	1.97
Rauwolfia vomitoria	Leaf	***	***	***	***	46.5	***	***	***
Ricinus communis	Whole plant	***	***	***	***	1.58	***	4.7	22.26
Sida cordifolia	Root	***	***	***	***	1.79	***	4.681	1.309

Cu	Fe	Hg	Mn	Ni	Pb	Se	V	Zn	Sample location	Reference
86.5	105	***	1,190	25	***	***	***	65.5	Ghana	Annan et al. (2010)
50.2	230	***	26	13.5	***	***	***	76.5	Ghana	Annan et al. (2010)
***	64.5	***	19.1	***	***	***	***	41.1	India	Garg et al. (2007)
4.05	255	***	34.1	***	***	***	***	27.5	India	Garg et al. (2007)
19.19	679.7	***	37	22.96	10.34	***	***	65.85	Pakistan	Jabeen et al. (2010)
3.5	135.5	***	55	***	***	***	***	56	Ghana	Annan et al. (2010)
8.38	409.4	***	32.64	4.09	5.12	***	***	31.64	Pakistan	Jabeen et al. (2010)
160.9	313.8	***	108.1	207	***	15.9	45.9	87.2	Nigeria	Obiajunwa et al. (2002)
188.1	404.4	***	796.3	222.1	***	40.1	28.8	127.6	Nigeria	Obiajunwa et al. (2002)
0.1	3.71	***	***	1.01	***	***	1.35	0.1	India	Narendhiraknnan et al. (2005)
172.8	269.3	***	39.3	196.6	***	9.7	***	98.7	Nigeria	Obiajunwa et al. (2002)
0.5	5.7	***	***	0.9	***	***	2.26	0.3	India	Narendhiraknnan et al. (2005)
4.74	63.2	***	43.6	***	***	***	***	40	India	Garg et al. (2007)
40.7	1,210	***	474	***	***	***	***	60	India	Garg et al. (2007)
10.312	539.4	***	9.6	12.69	1.98	***	***	48.29	India	Rajurkar and Damame (1997)
138.1	305.4	***	66.2	121.5	***	13	***	88.9	Nigeria	Obiajunwa et al. (2002)
36.752	9.2	263.7	32.77	308.6	3.91	***	***	119.84	India	Rajurkar and Damame (1997)
12.98	688.1	***	35.36	6.54	8.24	***	***	28.92	Pakistan	Jabeen et al. (2010)
122.5	470.5	***	102	***	***	***	***	63.5	Ghana	Annan et al. (2010)
44.5	127.5	***	49.5	***	***	***	***	80.5	Ghana	Annan et al. (2010)
12.2	923	***	161	***	***	***	1.74	56.5	India	Garg et al. (2007)
9.35	574	***	22.55	***	***	***	***	47.5	Ghana	Annan et al. (2010)
15.62	317.7	***	64.6	8.1	10.63	***	***	31.55	Pakistan	Jabeen et al. (2010)
1.309	113.5	***	28.63	12.09	1.36	***	***	51.63	India	Rajurkar and Damame (1997)

(continued)

Table 1 (continued)

Species	Dry Weight	Al	As	Au	Br	Cd	Cl	Co	Cr
Solanum arguivi	Fruit	***	***	***	3.4	***	***	***	***
Solanum indicum	Root	***	***	***	***	***	3070	2.373	***
Solanum xanthocarpum	Fruit/ root	***	***	***	***	1.387	3010	1.586	0.198
Spondias monbin	Leaf	***	***	***	13.5	***	***	***	***
Symplocos racemosa	Bark	1,080	***	***	13.6	***	1,910	0.15	1.2
Terminalia chebula	Fruit	730	***	***	3.39	***	3,520	0.54	1.28
Tinospora cordifolia	Leaf/fruit	420	***	0.00047	9.78	***	450	0.07	0.961
Tribulus terrestris	Fruit	***	***	***	***	0.873	6,860	3.878	0.582
Turraea heterophylla	Leaf	***	***	***	***	43.5	***	***	***
Ureria picta	Root	***	***	***	***	740.19	***	8.981	2.566
Vernonia amygdalina	Leaf	***	***	***	***	44.5	***	***	***
Vetiveria zizanioides	Root	***	***	***	***	1.062	***	5.699	1.449
Vinca rosea	Leaf	***	***	***	***	39.5	***	***	***
Vitex negundo	Leaf	1,910	***	0.00115	0.88	***	370	0.07	1.07
Voacanga africana	Leaf	***	***	***	***	29.5	***	***	***
Withania somnifera	Leaf/root	1,000	***	***	23.1	***	1,300	0.07	1.86
Woodfordia floribunda	Flower	***	***	***	***	2.523	***	5.248	0.707
Zanthoxylum xanthoxyloides	Leaf	***	***	***	***	48	***	***	***
Zingiber officinale	Rhizome	***	***	***	7.9	***	***	***	***

*** Data not available

Cu	Fe	Hg	Mn	Ni	Pb	Se	V	Zn	Sample location	Reference
24.5	321.7	***	31.3	4.1	***	***	***	29.7	India	Singh et al. (2010)
4.845	129.4	***	11.71	8.6	***	***	***	43.3	India	Rajurkar and Damame (1997)
9.909	126.2	***	11.7	1.59	11.89	***	***	68.18	India	Rajurkar and Damame (1997)
222.4	354.6	***	137.7	130.5	***	22.9	27.7	69.3	Nigeria	Obiajunwa et al. (2002)
4.1	233	***	191	***	***	***	3.04	26.7	India	Garg et al. (2007)
5.6	656	***	12.2	***	***	***	1.7	35.1	India	Garg et al. (2007)
4.23	277	***	47.3	***	***	***	***	13.5	India	Garg et al. (2007)
8.436	89.1	193.9	12.91	4.46	7.76	***	***	25.79	India	Rajurkar and Damame (1997)
15.5	391	***	23.5	***	***	***	***	66.5	Ghana	Annan et al. (2010)
22.508	158	779.7	15.48	13.42	15.79	***	***	41.35	India	Rajurkar and Damame (1997)
12.5	130.5	***	***	***	***	***	***	58.5	Ghana	Annan et al. (2010)
5.409	1178.4	202.8	41.41	10.43	22.49	***	***	18.64	India	Rajurkar and Damame (1997)
17.5	84.5	***	28.5	3.5	***	***	***	98.5	Ghana	Annan et al. (2010)
3.65	263	***	44.2	***	***	***	***	13.8	India	Garg et al. (2007)
25	193	***	556	***	***	***	***	70.5	Ghana	Annan et al. (2010)
9.5	231	***	17.4	***	***	***	***	43.6	India	Garg et al. (2007)
9.386	152.3	686.3	6.19	17.15	18.17	***	***	28.46	India	Rajurkar and Damame (1997)
36	***	***	54.5	***	***	***	***	48.5	Ghana	Annan et al. (2010)
94.9	1,219	***	367.9	108.1	***	8.2	21.7	43.8	Nigeria	Obiajunwa et al. (2002)

Table 2 Comparison of the number of tested species that have Pb, Cd and Hg levels that exceed the European Pharmacopoeia maximum permissible levels (MPL) and maximum content of the heavy metals (HMs) reported to exist in the plant species presented in Table 1

European pharmacopoeia MPL	Pb (5 μg/g)	Cd (0.5 μg/g)	Hg (1 μg/g)
No of plant species below MPL	06	02	0
No of plant species above MPL	21	44	10
Data not available	61	44	78

Table 3 Regulatory limit values established by WHO, FDA, and European Pharmacopoeia (Ph. Eur.) for metals in herbal drugs daily dietary

Metal	WHO/FDA permissible limit	Ph. Eur. permissible limit (Gasser et al. 2009)	RDA (in mg)	FAO/WHO PTWI (mg kg^{-1} b wt.)
Al	***	***	***	***
As	10	***	***	15
Au	***	***	***	***
Br	***	***	1.5–2.5	***
Cd	0.3	0.5	***	7
Cl	***	***	***	***
Co	***	***	***	***
Cr	***	***	0.02–0.2	***
Cu	***	***	1.0–3.0	***
Fe	***	***	10–18	***
Hg	1	0.1	***	5
Mn	***	***	1.0–5.0	***
Ni	***	***	0.13–0.4	***
Pb	10	5	***	25
Se	***	***	0.02–0.2	***
V	***	***	0.1–0.4	***
Zn	***	***	15	***

US RDA recommended daily dietary allowance, *PTWI* provisional tolerable weekly intake, *b wt.* body weight
*** Data not available

(including members of the Asteraceae, Brassicaceae, Caryophyllaceae, Cyperaceae, Cunouniaceae, Fabaceae, Flacourtiaceae, Lamiaceae, Poaceae, Violaceae, and Euphobiaceae) (Kramer 2010). However, the ecological and biological significance of metal hyperaccumulation is not well understood. Notwithstanding, it has been firmly established that some medicinal plants do hyperaccumulate heavy metals, viz., As, Co, Hg, and Pb, and these metals at some levels are highly hazardous and impinge upon human health, sometimes even at very low concentrations.

The level to which metals accumulate in plants is influenced by the physico-chemical properties of the soil in which the plants grow. Such properties include the bioavailability of the metallic element, characteristics of soil or sediments, pH level, exposure period, dispersion range, and presence or absence of other elements. If availability of metallic elements in soil/sediment is high, then some plants

Table 4 Concentrations of heavy metals that are accumulated in *Vetiveria zizanioides* roots and shoots (Truong 1999)

Heavy metal	Soil mg kg^{-1}	Shoot mg kg^{-1}	Root mg kg^{-1}	Shoot/root (%)
As	620	11.2	268	4.2
Cd	1.66	0.31	14.2	2.2
Cu	50	13	68	19
Cr	600	18	1,750	1
Pb	730	78.2	87.8	87
Hg	6.17	0.12	10.8	11
Ni	300	448	1,040	43
Se	74.3	11.3	24.8	46

absorb these metals into the root system and translocate them to aerial plant parts (Truong 1999).

Some grass species, e.g., *Vetiveria zizanioides* absorb a wide range of heavy metals, if such metals are present in soil (Table 4), and we believe that this species is the most impressive example of a potentially hazardous medicinal plant. In general, grasses are metal-tolerant plants (Rosselli et al. 2003), and are high accumulators of heavy metals (Pichtel and Salt 1998); hence, consuming them may be hazardous.

Curiously, *V. zizanioides,* which is a well-known grass species, is a potential candidate for treating cardiovascular diseases (Rajurkar and Damame 1997), owing to its bioactive properties. However, if this plant is collected from metal-contaminated sites, the metal levels which exist may produce adverse toxic effects on the consumer.

In conclusion, heavy metal contamination may alter the chemical composition of plants, and thereby seriously affect the quality and efficacy of the plant products produced by medicinal plant species (Zhu and Cullen 1995). If medicinal plant species are collected from industrial areas, there is a greater risk that the plants will be contaminated than if the species are collected from natural (pristine or rural) areas. Moreover, we urge those who harvest *V. zizanioides* to ensure that the collected samples are carefully analyzed for metallic element before being put to herbal medicinal use.

Some medicinal plants absorb higher amounts of metals selectively from soil than do others (Table 5). *Clematis gouriana,* used for the treatment of skin diseases, takes up very high Cd levels. Ingestion of even trace quantities of Cd can influence the physiology and health of individual organisms and threaten their distribution, reproduction and survival (Meena et al. 2010).

Woodfordia floribunda has been frequently used in the pharmaceutical industry for its wide range of bioactive compounds. Unfortunately, this plant also absorbs Pb. At high levels, human exposure to Pb will damage most organs and organ systems, including the central nervous system, kidneys, and circulatory system, which can lead to death (Lansdown and Yule 1986; Goldstein 1992). Some medicinal plants contain high proportions of As and Hg, which has resulted in the safety being questioned of certain mineral and herbomineral medicines that absorb these elements, e.g., *Elsholtzia communis* and *Ureria picta*.

In many African countries, *Alchornea cordifolia* is commonly used as a medicine for the treatment of bacterial, fungal, parasitic, and inflammatory disorders (Mangaa

Table 5 Heavy metal uptake potential of some medicinal plants that have therapeutically active uses

Metal	Concentrations (μg/g)	Species	Family	Use and reference source
Br	43.4	*Aframomum melegueta*	Zingiberaceae	Juice of young leaves is styptic, seeds contain an alkaloid, piperine; gingerol, paradol, shogaol and zingerone (Khare 2007)
Se	41.3			
Cu	247.8			
Ni	308.6	*Ocimum tenuiflorum*	Lamiaceae	The Ayurvedic Pharmacopoeia of India recommends the use of the leaf and seed in rhinitis and influenza; the seed used in psychological disorders, including fear-psychosis and obsessions (Khare 2007)
Co	14.9			
Al	2,470	*Andrographis paniculata*	Acanthaceae	Traditionally used for the treatment of colds, fever, laryngitis and several infectious diseases ranging from malaria to dysentery and diarrhea. The plant is widely used in Ayurvedic and Homeopathic systems of medicines (Mishra et al. 2010)
AS	2.1	*Elsholtzia communis*	Lamiaceae	Used as tonic, astringent, carminative and antiseptic. Decoction of leaves and flowers is given in tonsillitis, fever, cough, high blood pressure, nose bleeding and menstrual disorder (Singh et al. 2010)
Au	0.48	*Cassia rhombifolia*	Fabaceae	Anti-inflammatory and antioxidant activities (Ilavarasan et al. 2005)
Cd	864.91	*Clematis gouriana*	Ranunculaceae	Paste of leaves is applied topically on affected part of the skin (Ignacimuthu et al. 2006)
Cr	198.6	*Calotropis procera*	Asclepiadaceae	The Ayurvedic Pharmacopoeia of India indicated the use of the root and leaf for asthma and dyspnoea; stem bark used for diseases of the spleen (Khare 2007)
Fe	13,295	*Celastrus paniculata*	Celastraceae	Seed oil stimulates the intellect and sharpens memory, nerve tonic, rejuvenate, antidepressant, antioxidant, free-radical scavenger (Rekha et al. 2005)
Hg	779.7	*Ureria picta*	Fabaceae	Root used in cardiac disorder (Rajurkar and Damame 1997)
Mn	1,190	*Gymnema sylvestre*	Asclepiadaceae	Leaf -anti diabetic. Stimulates the heart and circulatory system, activates the uterus, and is used in parageusia and furunculosis. Plant-diuretic, antibilious. Root-emetic, expectorant, astringent, stomachic (Khare 2007)
Pb	18.17	*Woodfordia floribunda*	Lythraceae	A wide range of chemical compounds including tannins (especially those of the macrocyclic hydrolysable class), flavonoids, anthraquinone glycosides, and polyphenols have been isolated from this species and are useful for pharmacological treatment activities (Das et al. 2007)
V	339.9	*Alchornea cordifolia*	Euphorbiaceae	Used in the traditional medicine of many African countries for the treatment of bacterial, fungal, parasitic and inflammatory disorders (Mangaa et al. 2004)
Zn	7695.4	*Carica papaya*	Caricaceae	Ripe fruit-stomachic, digestive, carminative, diuretic, galactagogue. Useful in bleeding piles, haemoptysis, dysentery and chronic diarrhea. Seeds-emmengagogue, abortifacient, vermifuge. The seed juice is administered for enlarged liver and spleen, and for bleeding piles (Khare 2007)

et al. 2004). This species *absorbs* vanadium in leaves up to a level of 339.9 μg/g. In the past, vanadium compounds were prescribed as therapeutic agents for anemia, tuberculosis, and diabetes, but recent clinical results show that this element may show a broad spectrum of toxic effects on the respiratory, circulatory, and central nervous systems, digestive organs, kidneys, and skin (Venkataraman and Sudha 2005).

The utilization of metal-hyperaccumulator medicinal plants in herbal formulations is often risky and may produce imminent metal toxicity to sensitive consumers. Use of metal-contaminated medicinal plants points out the age-old conundrum, i.e., such plants may offer curative properties to multitudes of people, but metal contamination of these same plants may pose an ongoing risk to consumers, unless steps are taken to ensure that the contaminant levels are safe. Considering that market demand for herbal drug remedies has increased and will further increase worldwide, it is essential to refine procedures now used for premarket drug testing to ensure the quality, efficacy, and safety of such natural remedies. It is our opinion that sensible and state-of-the-art cultivation collection practices are essential for plants used in herbal medicines, and only this can provide the basis for appropriate quality assurance of medicinal plant use. Moreover, we believe the European and WHO guidelines for raw herbal material collections should be observed by all purveyors of plant medicines (WHO 2003, 2007; European Medicines Agency 2006).

6 Conclusions

The results of our literature review of heavy metal accumulation in medicinal plants vs. safe levels lead us to conclude the following:

1. Medicinal plants are prone to contamination from heavy metals.
2. The levels of heavy metals found in medicinal plants reviewed are generally low. However, excessive and potentially unsafe levels of several metals have appeared in the 88 species of marketable plant medicine species reviewed: Pb in 21 species, Cd in 44 species, and Hg in 10 species.
3. Authorities should establish a more standardized and universally accepted value for safe levels of heavy metals, metallic minerals or for metal content in medicinal plants.
4. Analyses are needed for both end products and raw materials that go to make phytopharmaceuticals on a priority basis.
5. Steps should be taken to prevent collection and marketing of such medicinal plants that are prone to heavy metal accumulations.

7 Summary

In this review, we evaluate the reports published between 1993 and 2011 that address the heavy metal accumulation in 88 medicinal plant species. We compare the safe limits for heavy metals set by governmental agencies vs. the levels at which such

metals actually exist in selected medicinal plants. We also evaluate the uses and effectiveness of medicinal plants in health care, and assess the hazards of medicinal plant uses, in view of the growing worldwide use of medicinal plants. From our extensive review of the literature, we discovered that a maximum permissible level (MPL) of Pb is exceeded in 21 plant medicine species, Cd in 44 species, and Hg in 10 species. *Vetiveria zizanioides* a potential candidate species for the treatment of cardiovascular diseases absorb a wide range of heavy metals from metal-contaminated soils. We believe that this species is the single most impressive example of a potentially hazardous medicinal plant.

Based on our review, we endorse the hypothesis that heavy metal accumulation by medicinal plants is mainly caused by extraction of soluble metals from contaminated soil, sediments and air. One continuing problem in protecting consumers of plant-based medicines is that permissible levels of all heavy metals in herbal medicine have not yet been standardized by regulating governmental entities. Moreover, there are few limit tests that exist for heavy metal content of medicinal plants, or permissible limits for essential dietary minerals, in most medicinal plants. The dearth of such limits hamstrings development of medicinal plant research and delays the release of either new or improved versions of medicinal plants or their components. In the present review, we emphasize that medicinal plants are often subjected to heavy metal contamination and that the levels at which these heavy metals sometimes occur exceeds permissible levels for some species. Therefore, collecting medicinal plants from areas that are, or may be, contaminated should be discouraged and banned if possible.

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References

Ajasa AO, Bello MO, Ibrahim AO, Ogunwande IA, Olawore NO (2004) Heavy trace metals and macronutrients status in herbal plats of Nigeria. Food Chem 85:67–71.

Annan K, Kojo AI, Cindy A, Samuel A, Tunkumgnen BM (2010) Profile of heavy metals in some medicinal plants from Ghana commonly used as components of herbal formulations. Phcog Res 2:41–44.

Arceusz A, Radecka I, Wesolowski M (2010) Identification of diversity in elements content in medicinal plants belonging to different plant families. Food Chem 120:52–58.

Arnold J (2001) Forestry, poverty and aid. Occasional paper No. 33. Center for international forestry research, Indonesia.

Ayodeji FB, Olorunsola OE (2011) Siam weed along highways, herbal medicine or poison? Toxicol Environ Chem 93:487–493.

Bernath J (2002) Preface. In: Bernath J, Zamborine Nemeth E, Craker, L Kock O (eds) International conference on medicinal and aromatic plants. Possibilities and limitations of medicinal and aromatic plant production in the 21st century. ISHS Acta Horticult 576, Budapest

Bhat R, Kiran K, Arun AB, Karim AA (2010) Determination of mineral composition and heavy metal content of some nutraceutically valued plant products. Food Anal Methods 3:181–187.

Bhat R, Sridhar KR (2008) Nutritional quality evaluation of electron beam-irradiated lotus (Nelumbo nucifera) seeds. Food Chem 107:174–184.
Bin C, Xiaoru W, Lee FSC (2001) Pyrolysis coupled with atomic absorption spectrometry for determination of mercury in Chinese medicinal materials. Analytica Chimica Acta 447: 161–169.
Blicharska E, Komsta L, Kocjan R, Gumieniczek A, Robak A (2010) A preliminary study on the effect of mineralization parameters on determination of metals in Viscum album species. Cent Eur J Chem 8:264–268.
Cantarelli MA, Pellerano RG, Del Vitto LA, Eduardo J, Marchevsky EJ, Camina JM (2010) Characterisation of Two South American Food and Medicinal Plants by Chemometric Methods based on their Multielemental Composition. Phytochem Anal 21:550–555.
Chaney RL, Malik M, Li YM, Brown SL, Brewer EP, Angle JS, Baker AJM (1997) Phytoremediation of soil metals. Curr Opin Biotech 8:279–284.
Chen ZS (1992) Metal contamination of flooded soils, rice plants and surface water in Asia. In: Adriano DC (ed) Biogeochemistry of trace metals. Lewis Publishers Inc, pp 85–93.
Chuparina EV, Aisueva TS (2011) Determination of heavy metal levels in medicinal plant Hemerocallis minor Miller by X-ray fluorescence spectrometry. Environ Chem Lett 9:19–23.
CIFOR (2002). Woodlands and rural livelihoods in dryland Africa, Indonesia. Center for International Forestry Research Info Brief 4:1–4.
Cobbet CS (2000) Phytochelatin biosynthesis and function in heavy metal detoxification. Curr Opin Plant Biol 3:211–216.
Commission Regulation (2001) Setting maximum levels for certain contaminants in foodstuffs. Official J European Communities, 466. http://www.caobisco.com/doc_uploads/legislation/466-2001EN.pdf
Cranton EM, Frackelton JP (1998) Free oxygen radical pathology and EDTA chelation therapy: mechanisms of action. J Adv Med 11:277–310.
Das PK, Goswamia S, Chinniaha A, Pandaa N, Banerjeea S, Sahua NP, Acharia B (2007) Woodfordia fruticosa: Traditional uses and recent findings. J Ethnopharmacol 110:189–199.
De Vas CHR, Schat H, De Waal MAM, Voojs R, Ernst WHO (1991) Increased ressistance to copper-induced damgae of root cells plasmalemma in copper tolerant Silence cucubalus. Physiol Plantarum 82:523–528.
Dhir B, Sharmila P, Saradhi P, Nasim SA (2009) Physiological and antioxidant responses of Salvinia natans exposed to Chromium-rich wastewater. Ecotoxicol Environ Saf 72:1790–1797.
Domany G, Barta-Szalai G, Gere A, Farsang G, Schon I (1996) 4-(1-Naphthylamino)-piperidines as inhibitors of lipid peroxidation. Arzneim-Forsch 46:553–556.
Donkin SG, Ohlson DL (2000) Teaf CM. Properties and effects of metals. In: Williams PL, James RC, Roberts SM (eds) Principles of Toxicology: Environmental and Industrial Applications, John Wiley and Sons Inc., New York, pp 325–45.
Elekes CC, Dumitriu I, Busuioc G, Iliescu NS (2010) The appreciation of mineral element accumulation level in some herbaceous plants species by ICP-AES method. Environ Sci Pollut Res 17:1230–1236.
Ernst E (2002) Heavy metals in traditional Indian remedies. Eur J Clin Pharmaco l 57:891–896.
Ernst WHO, Verkleij JAC, Schat H (1992) Metal tolerance in plants. Acta Botanica Neerlandica 41:229–248.
European Medicines Agency (2006) Guideline on Good Agricultural and Collection Practice for starting materials of Herbal Origin, EMEA/HMPC/246816/05, London.
FAO (2005) The state of food insecurity in the World: Eradicating world hunger- key to achieving the Millennium Development Goals, FAO, Rome.
Fox TC, Guerinot ML (1998) Molecular biology of cation transport in plants. Ann Rev Plant Physiol Plant Mol Biol 49:669–696.
Garg N, Kumar A, Nair AGC, Reddy AV (2007) Analysis of some Indian medicinal herbs by INAA. J Radioanal Nucl Ch 271:611–619.
Gasser U, Klier B, Kuhn AV, Steinhoff B (2009) Current Findings on the Heavy Metal Content in Herbal Drugs: Evaluation of a Database. Pharmeur Sci Notes 21:37–49.

Gjorgieva D, Kadifkova-Panovska T, Baceva K, Stafilov T (2010) Content of toxic and essential metals in medicinal herbs growing in polluted and unpolluted areas of Macedonia. Arh Hig Rada Toksikol 61:297–303.

Goldstein GW (1992) Neurological concepts of lead poisoning in children. Pediatr Ann 21:384–388.

Hay RW(1984) Bio-inorganic chemistry, Ellis Horwood Ltd, pp 1–397.

Hendler E, Sheldon S (1990) The Doctors' Vitamin and Mineral Encyclopedia, Simon and Schuster, New York. pp 1–496.

Herrera-Estrella LR, Guevara-Garcia AA (2009) Heavy Metal Adaptation. eLS Encyclopedia of Life Sciences. John Wiley & Sons, Ltd. Published Online: 15 MAR 2009, doi. 10.1002/9780470015902.a0001318.pub

Heyes RB (1997) The Carcinogenicity of metals in humans. Cancer Causes Control 8: 371–385.

Hsu M J, Selvaraj K, Agoramoorthy G (2006) Taiwan's industrial heavy metal pollution threatens terrestrial biota. Environ Pollut 143:327–334.

Ignacimuthu S, Ayyanar M, Sankara SK (2006) Ethnobotanical investigations among tribes in Madurai District of Tamil Nadu (India). J Ethnobiol Ethnomed 2:25. doi.10.1186/1746-4269-2-25.

Ilavarasan R, Mallika M, Venkataraman S (2005) Anti-inflammatory and antioxidant activities of cassia fistula linn bark extract. Afr J Trad CAM 2:70–85.

International Programme on Chemical Safety (2009) JECFA glossary of terms. http://www.who.int/ipcs/food/jecfa/glossary.pdf.

Ives AR, Cardinale B (2004). Food-web interactions govern the resistance of communities after non-random extinctions. Nature 429:174–177.

Ivey M, Elmen G (1986) Nutritional supplements, mineral and vitamin production in Handbook of nonprescription Drugs, 8th Ed. American Pharmaceutical Association, The National Professional Society of Pharmacists, Washington DC pp 1–215.

Jabeen S, Shah MT, Khan S, Hayat MQ (2010) Determination of major and trace elements in ten important folk therapeutic plants of Haripur basin, Pakistan. J Med Plant Res 4:559–566.

Khan MA, Ahmad I, Rahman I (2007) Effect of environmental pollution on heavy metals content of Withania somnifera. J Chinese Chem Soc 54:339–343.

Khare CP (2007) Indian Medicinal Plants: An Illustrated Dictionary, Springer-Verlag Berlin, Heidelberg, pp 1–836.

Koe H, Sari H (2009) Trace metal contents of some medicinal, aromatic plants and soil samples in the Mediterranean region. Turkey J Appl Chem Res, 8:52–57.

Kosalec I, Cvek J, Tomic S (2009) Contaminants of medicinal herbs and herbal products. Arh Hig Rada Toksikol 60:485–501.

Kramer U (2010) Metal Hyperaccumulation in Plants. Annu Rev Plant Biol 61:517–34.

Laloo RC, Kharlukhi L, Jeeva S, Mishra BP (2006) Status of medicinal plants in the disturbed and the undisturbed sacred forests of Meghalaya, northeast India: population structure and regeneration efficacy of some important species. Curr Sci 90:225–231.

Lansdown R, Yule W (1986) Lead toxicity: History and environmental impact, The Johns Hopkins University Press, Baltimore.

Larison JR, Likens E, Fitzpatrick JW, Crock JG (2000) Cadmium toxicity among wildlife in the Colorado rocky mountains. Nature 406:181–183.

Lekouch N, Sedki A, Nejmeddine A, Gamon S (2001) Lead and traditional Moroccan pharmacopoeia. Sci Total Environ 280:39–43.

Liang J, Wang QQ, Huang BL (2004) Concentration of hazardous heavy metals in environmental samples collected in Xiaman, China as determined by vapor generation non-dispersive atomic fluorescence spectrometry. Anal Sci 20:85.

Lozak A, Soltyk K, Ostapczuk P, Fijalek Z (2002) Determination of selected trace elements in herbs and their infusions. Sci Total Environ 289:33–40.

Maharia RS, Dutta RK, Acharya R, Reddy AVR (2010) Heavy metal bioaccumulation in selected medicinal plants collected from Khetri copper mines and comparison with those collected from fertile soil in Haridwa. India. J Environ Sci Heal B 45:174–181.

Maiga A, Diallo D, Bye R, Paulsen BS (2005) Determination of some toxic and essential metal ions in medicinal and edible plants from Mali. J Agric Food Chem 53:2316–2321.
Mangaa HM, Brkica D, Mariea DEP, Quetin-Leclercqa J (2004) In vivo anti-inflammatory activity of Alchornea cordifolia (Schumach. & Thonn.) Müll. Arg. (Euphorbiaceae). J Ethnopharmacol 92:209–214.
Meena AK, Kadirvelu K, Mishra GK, Rajagopal C, Nagar PN (2008). Adsorptive removal of heavy metals from aqueous solution by treated sawdust (Acacia arabica). J Hazard Mater 150: 604–611.
Meena AK, Bansal P, Kumar S, Rao MM, Garg VK (2010) Estimation of heavy metals in commonly used medicinal plants: a market basket survey. Environ Monit Assess 170:657–660.
Mertz W (1981). The essential trace elements. Science. 213:1332–1338.
Mishra S, Tiwari SK, Kakkar A, Pandey AK (2010) Chemoprofiling of Andrographis Paniculata (Kalmegh) for its Andrographolide Content in Madhya Pradesh, India. Int J Pharma Bio Sc 6: 1–5.
Narendhiraknnan RT, Subramanian S, Kandaswamy M (2005) Mineral content of some medicinal plants used in the treatment of diabetes mellitus. Biol Trace Elem Res 103:109–115.
Nasim SA, Dhir B (2010) Heavy metals alter the potency of medicinal plants. In: Whitacre DM(ed) Rev Environ Contam Toxicol, 203:139–149, doi 10.1007/978-1-4419-1352-4_5
Nriagu JO, Pacyna JM (1988) Quantitative assessment of worldwide contamination of air water and soils by trace metals. Nature 333:134–139.
Obi E, Akunyili DN, Ekpo B, Orisakwe OE (2006) Heavy metal hazards of Nigerian herbal remedies. Sci Total Environ 369:35–41.
Obiajunwa EI, Adebajo CA, Omobuwajo OR (2002) Essential and trace element contents of some Nigerian medicinal plants. J Radioanal Nucl Ch 252:473–476.
Oliver MA (1997) Soil and human health: A review. Eur J Soil Sci 48:573–592.
Pichtel J, Salt CA (1998) Vegetative growth and trace metal accumulation on metalliferous wastes. J Environ Qual 27:618–624.
Rajendran A, Narayanan V, Gnanavel I (2007) Study on the analysis of trace elements in Aloe vera and its biological importance. J Appl Sci Res 3:1476–1478.
Rajurkar NS, Damame MM (1997) Elemental analysis of some herbal plants used in the treatment of cardiovascular diseases by NAA and AAS. J Radioanal Nucl Ch 219:77–80.
Rekha K, Bhan MK, Balyan SS, Dhar AK (2005) Cultivation prospects of endangered species Celastrus paniculatus wild. Nat Prod Rad 4:482–486.
Rosselli W, Keller C, Boschi K (2003) Phytoextraction capacity of trees growing on a metal contaminated soil. Plant Soil 256:265–272.
Salgueiro L, Martins AP, Correia H (2010) Raw materials: the importance of quality and safety. A review. Flavour Fragr J 25:253–271.
Sarma H (2011) Metal Hyperaccumulation in Plants: A Review Focusing on Phytoremediation Technology J Environ Sc Technol 4: 118–138. doi.10.3923/jest.
Sarma H, Sarma CM (2008) Alien traditionally used plants species of Manas Biosphere Reserve, Indo-Burma hotspot. Zeitschrift Fur Arznei and Gewurzpflanzen 13:117–120.
Sarma H, Sarma AM, Sarma CM (2008) Tradycyjna wiedza o roslinach dziko rosnacych uzywanych jako warzywa i do celow leczniczych w regione barpeta (Prowincja Assam, Indie). Herba Pol 54:80–88.
Shamsa F, Zadeh SR, Shamsa H, Abdi K (2009) A Quantitative investigation on some toxic and non-toxic metals in popular medicinal herbs in Iranian market Iran J Pharm Res 8: 95–99.
Sharma KR, Agrawal M, Marshall MF (2009) Heavy metals in vegetables collected from production and market sites of a tropical urban area of India. Food Chem Toxicol 47:583–591.
Sheded GM, Pulford ID, Hamed IA (2006) Presence of major and trace elements in seven medicinal plants growing in the South-Eastern Desert. Egypt J Arid Env 66:210–217.
Singh NKS, Devi CB, Singh TS, Singh NR (2010) Trace elements of some selected medicinal plants of Manipur. Indian J Nat Prod Resour 1:227–231.
Tolonen M (1990) Vitamins and minerals in health and nutrition, Ellis Horwood Ltd, Chichester, pp 148–187.

Truong P (1999) Vetiver Grass Technology for Mine Rehabilitation. Pacific Rim Vetiver Network Tech Bull 2.
Venkataraman BV, Sudha S (2005) Vanadium Toxicity. Asian J Exp Sci 19:127–134.
Wang CF, Duo MJ, Chanc HE, Yang JY (1985) Essential and toxic trace elements in Chinese medicine. J Radioanal Nucl Chem 211:333–347.
Wong MK, Tan P, Wee YC (1993) Heavy metals in some of Chinese herbal plants. Biol Trace Elem Res 36:135–142.
World Bank (2001) World Development Report 2000/2001, Attacking Poverty Oxford, Oxford University Press, pp 1–200.
World Bank (2004) Sustaining forests: A development strategy, World Bank, Washington DC.
WHO (2002) Traditional Medicine Strategy 2002–2005, WHO, Geneva.
WHO (2003) WHO guidelines on good agricultural and field collection practices (GACP) for medicinal plants, WHO, Geneva.
WHO (2005) Quality Control Methods for Medicinal Plant Materials, Revised, WHO, Geneva.
WHO (2007) WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residue, WHO, Geneva.
Zhu L, Cullen WR (1995) Effects of some heavy metals on cell suspension cultures of Catharanthus roseus. J Environ Sci 7:60–65.

Effects of Organic Herbicides on Phototrophic Microbial Communities in Freshwater Ecosystems

Stéphane Pesce, Agnès Bouchez, and Bernard Montuelle

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1 Introduction

Pollution of aquatic ecosystems by pesticide contamination is a major environmental concern. Numerous authors have addressed the frequent occurrence of chronic or acute herbicide contamination of freshwater ecosystems in both agricultural and urban areas of the world (Devault et al. 2007; Gilliom 2007; Schuler and Rand 2008; Woudneh et al. 2009). The physiological characteristics of photosynthetic microorganisms make them attractive as targets for herbicides in aquatic ecosystems. Since these primary producers form the basis of trophic structure in many aquatic environments, herbicides may threaten the entire equilibrium of the ecosystems they contaminate.

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The tests that are most widely used to assess the toxicity of herbicides on autotrophic microorganisms (especially microalgae) are monospecific toxicity tests; such tests combine low cost with satisfactory reproducibility and ease of execution (Seguin et al. 2001). The results of single-species toxicity tests with algae have produced large herbicide-dependent sensitivity differences (Table 5.1; DeLorenzo et al. 2001). However, discernment is required when extrapolating results from monospecific assays to ecosystem impairment, and many authors have cited the importance of reinforcing the ecological relevance of toxicological studies to improve ecotoxicological risk assessment (Chapman 2002; Relyea and Hoverman 2006; Filser 2008; Schmitt-Jansen et al. 2008). A first step in such reinforcement is to evaluate toxic effects at the community level by applying community ecology concepts to ecotoxicology testing (Schmitt-Jansen et al. 2008; Clements and Rohr 2009; Geiszinger et al. 2009).

On the basis of the foregoing considerations, the purpose of this paper is as follows:

1. To provide a broad bibliographical review of experimental and in situ studies performed over the last 15 years that address the effects of herbicides, either alone or in pesticide mixtures, on free and attached autotrophic microbial communities
2. To identify potential research areas that can benefit from future research

2 Experimental Studies

2.1 Effects of Single Herbicides

2.1.1 Triazines

Atrazine

The effects of atrazine on freshwater phototrophic microorganisms have been widely studied over the past 15 years (Table 5.2; Solomon et al. 1996; DeLorenzo et al. 2001).

Chronic Effects on Biomass and Primary Production

Concentrations of chlorophyll *a* (chl *a*) are generally used to estimate the biomass of photosynthetic microorganisms. Many authors have observed a decrease in phytoplankton chl *a* (Seguin et al. 2002; Perschbacher et al. 2008) or periphyton chl *a* (DeLorenzo et al. 1999; Nyström et al. 2000; Seguin et al. 2002; Downing et al. 2004; Rohr and Crumrine 2005; Schmitt-Jansen and Altenburger 2005a; Guasch et al. 2007) following atrazine exposure at concentrations ranging from 20 to 1,000 µg/L. At lower concentrations, the effects of atrazine on algal biomass are more variable (Table 5.2). For example, chl *a* concentrations in phytoplankton (van den Brink et al. 1995; Leboulanger et al. 2001; Relyea 2009) and periphyton (Gruessner and Watzin 1996; Muñoz et al. 2001) were not affected by atrazine

Table 1 Main characteristics of the herbicides cited in this review (source: Footprint Database; http://www.eu-footprint.org/)

Herbicide	Class	Koc (ml g^{-1}) (mL/g)	Wat-Sed DT50 (Days)	Water DT50 (Days)	Growth test			
					EC50-72 h (mg/L)		NOEC-96h (mg/L)	
2,4-D	Alkylchlorophenoxy	56	29	29	24.2	*Raphidocelis subcapitata*	100	*Chlorella vulgaris*
Acetolachlor	Chloroacetamide	156	19.7	40.5	0.00027	*Pseudokirchneriella subcapitata*	0.00059	*Selenastrum capricornutum*
Alachlor	Chloroacetamide	124	2	–	0.966	*Scenedesmus quadricauda*	0.02	*Chlorella pyrenoidosa*
Asulam	Carbamate	–	–	–	–	–	–	–
Atrazine	Triazine	100	80	–	0.059	*Raphidocelis subcapitata*	0.1	Green algae
Bensulfuron-methyl	Sulfonylurea	315	48	18.5	0.02	*Raphidocelis subcapitata*	–	–
Chlorsulfuron	Sulfonylurea	36.3	26	21	0.068	*Pseudokirchneriella subcapitata*	–	–
Clomazone	Isoxazolindinone	287	54	–	0.136	*Navicula pelliculosa*[a]	0.05	*Navicula pelliculosa*[a]
Diuron	Phenylurea	1,067	48	8.8	0.0027	*Scenedesmus quadricauda*	–	–
Fomesafen	Diphenyl ether	50	7	–	0.17	*Unknown species*	–	–
Glufosinate-ammonium	Phosphinic acid	755	24.5	7.2	46.5	*Scenedesmus quadricauda*	320	Unknown species
Glyphosate	Phosphonoglycine	21,699	87	2.5	4.4	*Scenedesmus quadricauda*	2	Unknown species
Halosulfuron-methyl	Pyrazole	115	–	–	0.0053	*Pseudokirchneriella subcapitata*	–	–
Hexazinone	Triazine	54	–	–	0.0145	*Pseudokirchneriella subcapitata*	–	–
Irgarol[b]	Triazine	–	–	–	–	–	–	–

(continued)

Table 1 (continued)

Herbicide	Class	Koc (ml g^{-1}) (mL/g)	Wat-Sed DT50 (Days)	Water DT50 (Days)	Growth test			
					EC50-72 h (mg/L)		NOEC-96h (mg/L)	
Isoproturon	Phenylurea	122	149	40	0.013	*Navicula pelliculosa*	0.052	Unknown species
Linuron	Phenylurea	620	46	48	0.016	*Raphidocelis subcapitata*	0.01	Unknown species
Metamitron	Triazine	80.7	11.1	10.5	0.4	*Pseudokirchneriella subcapitata*	0.1	Unknown species
Metazachlor	Chloroacetamide	134	20.6	216	0.0162	*Pseudokirchneriella subcapitata*	0.34	Unknown species
Methabenzthiazuron	Urea	527	182	90	0.033	*Scenedesmus quadricauda*	0.018	Unknown species
Metolachlor	Chloroacetamide	200	365	88	57.1	*Pseudokirchneriella subcapitata*	–	–
Metribuzin	Triazinone	38	50	41	0.02	*Scenedesmus subspicatus*	0.019	Unknown species
Metsulfuron methyl	Sulfonylurea	39.5	140	115	0.045	*Raphidocelis subcapitata*	0.02	Unknown species
Molinate	Thiocarbamate	190	61	4	0.5	*Raphidocelis subcapitata*	–	–
Nicosulfuron	Sulfonylurea	21	41.5	65	7.8	*Anabaena flos-aquae*	100	Unknown species
Pendimethalin	Dinitroaniline	15,744	16	4	0.006	*Raphidocelis subcapitata*	0.003	*Pseudokirchneriella subcapitata*
Pretilachlor	Chloroacetamide	–	–	–	9.29	*Scenedesmus acutus*	–	–
Profoxydim	Cyclohexadione oxime	–	47.4	35.6	33	*Anabaena flos-aquae*	–	–
Prometryn	Triazine	400	38	56	0.002	*Scenedesmus acutus*	–	–
Propanil	Anilide	400	–	–	0.05	*Unknown species*	–	–
Propazine	Triazine	154	77	77	0.18	Cyanobacteria	–	–

Prosulfocarb	Thiocarbamate	1,693	214	1.05	0.049	*Pseudokirchneriella subcapitata*	–	–
Quinclorac	Quinolinecarboxylic acic	50	–	–	6.53	*Scenedesmus acutus*	–	
Simazine	Triazine	130	33	46	0.04	*Scenedesmus subspicatus*	0.6	Unknown species
Simetryn	Triazine	200	–	–	0.0098	*Anabaena flosaquae*	–	–
Thiobencarb	Thiocarbamate	1,062	94.9	4.4	0.017	*Raphidocelis subcapitata*	–	–
Triallate	Thiocarbamate	4,301	57.4	104	0.0022	*Pseudokirchneriella subcapitata*	0.032	Unknown species
Triclopyr	Pyridine compound	48	29.2	24.8	75.8	*Raphidocelis subcapitata*	8	Unknown species

Wat-Sed water-sediment, DT_{50} 50% disappearance time, EC_{50} 50% effective concentration, *NOEC* no observed effect concentration

[a]Duration of test: 120 h

[b]Not referenced in the footprint database

"–" data not available in the database

Table 2 Summary list of studies in which the effects of atrazine were assessed on phototrophic freshwater microbial communities in experimental systems

Experimental system	Conc. (µg/L)	Community	Parameters	Effects	LOEC	Reference	Comments
5-L microcosms (10–21 days)	10	Phytoplankton	Community composition	+		Bérard et al. (1999a, b)	Seasonal variations in the response
			Primary production	±			
5-L microcosms	10	Phytoplankton	Community composition	+		Bérard and Benninghoff (2001)	Seasonal variations in the response
			PICT	±			
Laboratory streams (4 weeks)	12; 150	Periphyton	Biovolumes	+	12	Carder and Hoagland (1998)	
			Community compositon	–			
			Chlorophyll *a*	+	50		
4-L microcosms (72 h)	50; 250	Periphyton	Biovolumes	+	50	DeLorenzo et al. (1999)	
			Primary producton	+	50		
			Community composition	+	50		
			Chlorophyll *a*	–			
			Primary production	+	15		Effects on community composition
Wetland mesocosms (2–106 days)	15–75	Periphyton	Respiraton	+	25	Detenbeck et al. (1996)	Vary with nutrient supply ratio
			Community composition	±			
			PICT	+	50		
			Chlorophyll *a*	+	20		
0.6-L microcosms (168 h)	20; 200	Periphyton	Primary production	+	200	Downing et al. (2004)	
			Community composition	+	20		
120-L microcosms (14 days)	5	Periphyton	Chlorophyll *a*	–		Gruessner and Watzin (1996)	
			Chlorophyll *a*	+			

10-L microcosms (3 weeks)	100	Periphyton	Primary production	–		Guasch et al. (2007)	No influence of P addition on atrazine toxicity
			PICT	+			
			Chlorophyll a	–			
Lake enclosures	1-20	Phytoplankton	Community composition	+		Gustavson and Wängberg (1995)	
			PICT	–			
		Periphyton	PICT	–			
1,000-L pond mesocosms (6 weeks)	5–318	Phytoplankton	Community composition	+	182	Jüttner et al. (1995)	
			Primary production	+		Knauert et al. (2008, 2009)	
10,000-L pond mesocosms (96 or 173 days)	70	Phytoplankton	Community compositon	+		Knauer et al. (2010)	
			PICT	+			
			Chlorophyll a	–			
5-L microcosms (11 days)	10	Phytoplankton	Community composition	+		Leboulanger et al. 2001	
			Chlorophyll a	–			
Indoor channels (18 days)	14	Periphyton	Biovolumes	–		Muñoz et al. (2001)	Effects of atrazine are only
			Primary production	±			Detected under grazing pressure
			Community composition	±			
			Chlorophyll a	+	50		

(continued)

Table 2 (continued)

Experimental system	Conc. (µg/L)	Community	Parameters	Effects	LOEC	Reference	Comments
			Primary production	+	50		
22-L microcosms (4 weeks)	12–700	Periphyton	Sulfolipid synthesis	+	115	Nyström et al. (2000)	
			Community composition	+	21		
			PICT	+	12		
0.5-L microcosms (7 weeks)	0.1–10	Phytoplankton	Primary production	+	0.1	Pannard et al. (2009)	Influence of phosphorus fluctuations
			Community composition	+	0.1		On atrazine efffects
Outdoor artificial streams (29 days)	3–300	Periphyton	Primary production	+	100	Pearson and Crossland (1996)	
500-L mesocosms (7 days)	1.13–113	Phytoplankton	Chlorophyll a	+	113	Perschbacher et al. (2008)	
			Primary production	+	113		
2.5-L flasks (24–48 h)	25	Phytoplankton	Community composition	–		Pinckney et al. (2002)	
1,300-L pond mesocosms (35 days)	10	Phytoplankton	Chlorophyll a	–		Relyea (2009)	
		Periphyton	Biomass	–			
11-L mesocosms (4 weeks)	25	Periphyton	Chlorophyll a	+		Rohr and Crumrine (2005)	Assessment of cascading trophic effects
			Chlorophyll a	+	500		
20-L microcosms (14 days)	7.5–1,000	Periphyton	Community compositon	+	30	Schmitt-Jansen and Altenburger (2005a)	Comparison with SSD
			PICT	+	125		

5-L microcosms	10	Phytoplankton	Chlorophyll a	±		
			Community composition	+		Seguin et al. (2001)
5,000-L outdoor mesocosms (2 months)	2; 30	Phytoplankton	Chlorophyll a	−		
			Community composition	+	2	
			Chlorophyll a	+		
5,000-L pond mesocosms (25 days)	30	Phytoplankton	Community composition	+		Seguin et al. (2002)
			PICT	+		
600-L pond mesocosms (6 weeks)	5	Phytoplankton	Chlorophyll a	−		van den Brink et al. (1995)
			Community compositon	−		

LOEC low observed effect concentration, + significant effects (negative of positive), ± variable effects, − no effect, *PICT* pollution-induced community tolerance

concentrations ranging from 5 to 14 μg/L, while Gustavson and Wängberg (1995) observed that 1–20 μg/L atrazine increased phytoplankton biomass in lake enclosures after 2 weeks of exposure. Similarly, Seguin et al. (2001) showed that phytoplankton chl *a* concentrations were sometimes higher or lower in outdoor microcosms that were contaminated by 10 μg/L of atrazine. Atrazine effects on primary production also varied considerably across studies (Table 5.2), and sometimes varied by season (Bérard and Benninghoff 2001) or were affected by trophic interactions (e.g., presence of grazers; Muñoz et al. 2001). These results agreed with those of Detenbeck et al. (1996), who showed in wetland mesocosms that atrazine effects on periphyton differed from a priori predictions that were based on laboratory bioassay data. The reason given was that abiotic parameters, such as temperature or nutrients, grazing intensity and biotic relationship between organisms, had influenced outcomes.

Chronic Effects on Community Composition

A series of experimental studies were conducted with atrazine on natural communities from Lake Geneva. Results of these studies revealed that atrazine (10 μg/L) consistently acted to restructure the autotrophic community, by modifying species composition (Bérard et al.1999a, b; Bérard and Benninghoff 2001; Leboulanger et al. 2001; Seguin et al. 2001). Chlorophytes (especially *Chlorella vulgaris*) were usually more sensitive, and diatoms and cryptophytes were more tolerant to atrazine, whereas some species, such as the cyanobacterium *Oscillatoria limnetica*, exhibited a variable response to atrazine that depended on seasons and species interactions (Bérard et al. 1999a, b).

The high sensitivity that chlorophytes had to atrazine was also observed to occur in phytoplankton (Pannard et al. 2009) and periphyton (Downing et al. 2004) assemblages. By contrast, diatoms were often described as being the most tolerant taxa to atrazine effects (Jüttner et al. 1995; DeLorenzo et al. 1999; Downing et al. 2004; Schmitt-Jansen and Altenburger 2005a). However, atrazine effects on community composition were not always detectable in either phytoplankton (van den Brink et al. 1995; Pinckney et al. 2002) or periphyton (Carder and Hoagland 1998). Moreover, in the absence of grazing pressure by snails, Muñoz et al. (2001) found no difference in taxonomic composition between unexposed periphyton, or those exposed to 14 μg/L atrazine for 18 days. However, they did note that atrazine toxicity increased with grazing, and the main effects detected were on algal community structure. The authors divided algal taxa into four classes, based on physiognomy, and concluded that the interaction of atrazine and grazing caused a significant decrease in prostrate growth and filamentous forms that were the most sensitive to atrazine.

Pollution-Induced Community Tolerance (PICT) Assessment

Atrazine-induced tolerance in phytoplankton communities occurs, but the results have been variable among experiments. Bérard and Benninghoff (2001) and Seguin et al. (2002) detected a rapid increase in atrazine tolerance in phytoplankton communities that were exposed to 10 and 30 μg/L atrazine, respectively. Nyström et al. (2000) also observed atrazine-induced tolerance in periphyton exposed to

concentrations that ranged between 12 and 125 μg/L. At higher concentrations, such exposure produced severe community damage characterized by reduced algal species number and abundance. By contrast, Gustavson and Wängberg (1995) reported no tolerance induction in phytoplankton and periphyton communities exposed to 20 μg/L atrazine for 20 days, and Detenbeck et al. (1996) observed that periphyton developed atrazine resistance only at a level equal to or exceeding 50 μg/L. According to Guasch et al. (2007), phosphate concentration is not a parameter that affects atrazine tolerance induction in phototrophic communities.

Interestingly, Schmitt-Jansen and Altenburger (2005a) observed similar ranges of sensitivity by comparing test results from a PICT approach with atrazine and single-species toxicity data (species sensitivity distribution approach; SSD), despite the fact that both approaches utilized test systems of different complexity. The authors emphasized that their SSD approach was facilitated by the existence of a large and comprehensive atrazine toxicity dataset on various algal species.

Irgarol

Combining short-term bioassay and nanocosm experiments, Nyström et al. (2002) and Bérard et al. (2003) showed that the triazine herbicide Irgarol 1051 was more toxic to Lake Geneva phytoplankton and periphyton than was atrazine. Effects were observed on phytoplankton photosynthetic activity (short-term effects) and diversity (long-term effects after 5–24 days of exposure) from exposure to this herbicide at a level of less than 1 μg/L. Mohr et al. (2008a) also observed effects on planktonic and periphytic algal communities at exposure concentrations between 0.04 and 5 μg/L.

Nyström et al. (2002) and Bérard et al. (2003) concluded that chlorophytes, especially *Chlorella vulgaris*, were the most Irgarol-sensitive in natural assemblages. By contrast, studying highly contaminated ponds (1 and 5 μg/L), Mohr et al. (2008a) observed a decrease in diatoms and an increase in chlorophytes and cyanobacteria. Given these differences among studies, the authors went on to suggest that Irgarol does not trigger a group-specific response, but rather induced a species-level response.

Mohr et al. (2008a) also revealed that recovery processes vary greatly among free and fixed communities. Indeed, in the same study, the phytoplankton rapidly recovered from a pronounced breakdown immediately after Irgarol exposure, whereas periphytic communities showed no recovery within 150 days after treatment in ponds contaminated by 1 and 5 μg/L Irgarol. This suggests that the sorption of Irgarol on periphyton may have prolonged the exposure duration in the tested communities.

Other Triazines

Short-Term Effects

Brown and Lean (1995) performed bioassays on mesotrophic lake phytoplankton communities using several triazine herbicides (atrazine, simazine, propazine, and prometryn). Atrazine and propazine exerted the highest toxic effects on phosphate and ammonium uptake rates, respectively, whereas prometryn was the most toxic for

photosynthetic activity, which was measured by the [^{14}C]bicarbonate assimilation rate. Schmitt-Jansen and Altenburger (2005a) confirmed that prometryn is more toxic to periphytic communities than is atrazine in short-term inhibition tests of photosynthesis.

Chronic Effects and Recovery Processes

Fairchild and Sappington (2002) conducted a 6-week study in outdoor mesocosms and observed no statistically significant effect of metribuzin on periphyton biomass at concentrations up to 75 μg/L. Similarly, Brock et al. (2004) showed that metribuzin, at nominal concentrations less than or equal to 56 μg/L, had only mild and transient effects on phytoplankton and periphyton, and recovery occurred within 8 weeks. Long-term effects, lasting longer than 8 weeks, were only found in the 180 μg/L enclosures. In these two experiments, the absence of effects at lower concentrations may have resulted from the rapid dissipation rate of metribuzin in water (half-life of 5–9 days). Brock et al. (2004) found that another triazine herbicide (metamitron; 14–4,480 μg/L) was even less persistent in the water column (half-life of 1–3 days). Enclosure experiments with metamitron revealed treatment-related effects for photoautotrophic communities only at the two highest concentrations (i.e., 1,120 and 4,480 μg/L), followed by a fast recovery, thought to derive from the agents short dissipation half-life in water.

When the long-term effects produced by metribuzin and metamitron were compared to data from standard toxicity tests, in which an assessment factor was applied (first-tier approach; i.e., Lowest Observed Effect Concentrations; LOEC), and an SSD approach was used, Brock et al. (2004) concluded that these two assessment procedures proved highly protective, since they did not account for dissipation rate or recovery processes in complex ecosystems.

Gustavson et al. (2003) also recommended that exposure duration be considered when assessing herbicide effects on periphyton communities. Indeed, these authors observed that the effect concentration of metribuzin decreased by one to two orders of magnitude when exposure time increased from 1 to 2 to 24 h. The effect of exposure duration was even more significant for hexazinone. Hexazinone stimulated photosynthesis at the three lowest test concentrations (i.e., 0.4, 2, and 10 μg/L) after a 1-h exposure, whereas the stimulation disappeared after 24 h. Moreover, Gustavson et al. (2003) observed a recovery of photosynthetic activity within stream periphyton communities that were exposed to metribuzin for a period up to 48 h, following exposure for 48 h in herbicide-free water. Photosynthetic activity recovered even at the highest concentration (i.e., 50 μg/L), whereas photosynthesis suffered an 80% inhibition. Comparable recovery processes that occurred within 24 h after herbicide addition ended were observed by Schneider et al. (1995) for stream periphytic communities exposed to 145–432 μg/L hexazinone.

PICT Assessment

Measures of photosynthetic activity showed that an induced-tolerance existed in communities chronically exposed to prometryn concentrations of 2.5 μg/L and higher (Altenburger 2005a). Diatom species, especially *Nitszchia* sp., clearly became predominant following long-term exposure to higher test concentrations

(i.e., 160 and 320 µg/L), which suggests their high tolerance to prometryn. Similarly, Chang et al. (2011) and Kasai and Hanazato (1995a, b) reported high tolerance of diatom species to another triazine herbicide, simetryn. Kasai and Hanazato (1995b) isolated algal strains from nontreated and treated microcosms that had been exposed for at least 35-days. The authors investigated genetic changes that occurred following simetryn exposure. The most significant finding concerned the chlorophyta *Scenedesmus gutwinskii* var. *heterospina*, which exhibited a tolerance level 26–57 times higher for strains preexposed to simetryn than for controls strains. Interestingly, the authors showed that the isolated strains maintained their tolerance for nearly 2 years in the absence of simetryn, confirming the importance of genetic adaptation in tolerance induction, within exposed photoautotrophic communities.

2.1.2 Phenylureas

Diuron

Short-Term Effects

Brown and Lean (1995) performed a short-term bioassay to test the toxicity of 16 pesticides (including 14 herbicides) to lake phytoplankton. They demonstrated that diuron was the most toxic substance to photosynthetic activity (see Table 5.3). For example, using the same biological end-point, they found that another phenylurea herbicide, monuron, was about 20-fold less toxic than was diuron. Francoeur et al. (2007) observed drastic adverse effects of a 20 µM diuron level (i.e., 4.66 mg/L) on periphyton photosynthesis after only 5 min of exposure.

Chronic Effects and Recovery Processes

A negative diuron exposure effect was revealed in several studies on chl *a* levels and on primary production in both phytoplankton (Perschbacher and Ludwig 2004; Knauert et al. 2008, 2009; Knauer et al. 2010) and periphyton (McClellan et al. 2008; Tlili et al. 2008, 2010; Ricart et al. 2009; López-Doval et al. 2010) communities (Table 5.3). Diuron can impact these parameters at exposure concentrations even lower than 0.1 µg/L, within a few weeks of initial contact (McClellan et al. 2008; Ricart et al. 2009). Nevertheless, Tlili et al. (2010) demonstrated that the effects of diuron (10 µg/L for 3 weeks) on photosynthetic activity can be inhibited when water contains high PO_4^{3-} concentrations. It has also been shown that a 21-day exposure to 10 µg/L of diuron inhibited the development of phototrophic communities, whereas they bloomed in untreated microcosms (Pesce et al. 2006).

Phototrophic community composition can also be affected by chronic diuron exposure (Table 5.3), although results vary greatly among various studies. Perschbacher and Ludwig (2004) showed that phytoplankton community composition was impacted by diuron (at 2 and 20 µg/L): cyanobacteria were severely reduced, while diatom and green algae were stimulated. Conversely, McClellan et al. (2008) and Tlili et al. (2010) observed a decrease in the relative number of diatoms in periphyton communities that were exposed to diuron concentrations

Table 3 Summary list of studies in which the effects of diuron were assessed on phototrophic freshwater microbial communities in experimental systems

Experimental system	Conc. (µg/L)	Community	Parameters	Effects	LOEC	Reference	Comments
Short-term bioassays (2 h)	4,662	Periphyton	Primary production	+		Francoeur et al. (2007)	
10,000-L pond mesocosms (96 or 173 days)	5	Phytoplankton	Primary production	+		Knauert et al. (2008, 2009)	
			Community composition	+			
			PICT	+		Knauer et al. (2010)	
20-L microcosms (5 days)	2.2; 11	Phytoplankton	Chlorophyll *a*	−		Leboulanger et al. (2011)	
			Biovolumes	+	2.2		
			Community composition	−			
Indoor channels (29 days)	2	Periphyton	Chlorophyll *a*	−		López-Doval et al. (2010)	No effect interaction between diuron and grazers
			Primary production	+			
15-L microcosms (11–21 weeks)	0.0032–50	Periphyton	Primary production	+	0.08	McClellan et al. (2008)	
			Communtiy composition	+	0.08		
			PICT	+	0.08		
500-L mesocosms (28 days)	0.2–20	Phytoplankton	Chlorophyll *a*	+	2	Perschbacher and Ludwig (2004)	
			Primary production	+	2		
			Community composition	+	2		
5-L microcosms (21 days)	10	Phytoplankton periphyton	Chlorophyll *a*	+		Pesce et al. (2006)	
			Community composition	+			
indoor channels (29 days)	0.07–7	Periphyton	Chlorophyll *a*	+	0.07	Ricart et al. (2009)	
			Primary production	+	0.2		
			Biovolumes	+	0.07		
			Community composition	+	0.07		

35-L microcosms (28 days)	1 (± pulses 7 or 14)	Periphyton	Chlorophyll *a* Primary production Community composition PICT	+ + + –	Tlili et al. (2008)	Effects of acute pulses varied according to the biological parameters tested
35-L microcosms (21 days)	10	Periphyton	Chlorophyll *a* Primary production Community composition PICT	± ± + +	Tlili et al. (2010)	Effects of diuron on chlo a and photosynthetic efficiency were modulated by P concentrations

LOEC low observed effect concentration, + significant effects (negative of positive), ± variable effects, – no effect

between 0.02 and 10 μg/L. Ricart et al. (2009) also observed changes in diatom composition of periphyton that were exposed for 29 days to low concentrations of diuron. Their work showed that the most sensitive end point was diatom biovolumes, which significantly decreased in the presence of diuron within 8 days, even in the treatments receiving the lowest concentration (i.e., 0.07 μg/L). This result was in accordance with that of Leboulanger et al. (2011), who reported a decrease in phytoplankton biovolumes following a 5-day exposure to 2.2 and 11 μg/L diuron.

Using three successive treatments of 5 μg/L of diuron, Knauert et al. (2009) observed that the herbicide significantly affected phytoplankton density and diversity, during 5 weeks of constant exposure. The most sensitive species were the cryptophyceae *Chroomonas acuta* and *Cryptomonas erosa* et *ovata*. Diuron exhibited a dissipation half-life of 43 days, allowing the phytoplankton community to recover both abundance and diversity during the 33–173 day posttreatment period.

Chronic Versus Acute Effects

Tlili et al. (2008) assessed the response of chronically contaminated biofilms (32 days, 1 μg/L diuron) to short pulses of diuron exposure (3 h; 7 and 14 μg/L). They detected several effects, including a significant increase in chl *a* fluorescence in periphyton chronically exposed to 1 μg/L diuron, increases in biomass and photosynthetic carbon incorporation, and changes in algal community structure (assessed by Polymerase Chain Reaction-Denaturing Gradient Gel Electrophoresis (PCR-DGGE) on 18S rDNA gene fragment and pigment analysis). Diuron pulses (single or double pulses at 7 or 14 μg/L) inhibited carbon incorporation in all biofilm communities, especially in the control microcosm. Nevertheless, the different pulses only affected community composition in control biofilms, revealing that the impact on a biofilm of a pulsed acute exposure to diuron depends on whether communities had previously been exposed to this herbicide.

PICT Assessment

McClellan et al. (2008) observed an increase in community tolerance for long-term concentrations of 0.08–10 μg/L diuron, whereas a chronic exposure of 50 μg/L was intolerable for periphyton, which was severely disturbed. Interestingly, the authors emphasized the fact that the observed threshold concentration of 0.08 μg/L, which caused effects on periphyton biomass and composition, as well as a shift in community tolerance, could not be predicted by extrapolation methods such as SSD or acute-to-chronic effect ratios.

An increase in periphtyon tolerance was also recorded by Tlili et al. (2010), following a 3-week exposure to 10 μg/L diuron. Their work revealed that phosphate concentration didn't influence diuron tolerance induction in the exposed communities as Guasch et al. (2007) had shown with atrazine. Nevertheless, Tlili et al. (2008) did not detect PICT in periphyton exposed for 32 days to 1 μg/L diuron. According to the authors, the lack of PICT processes could be due to the regular supply of nonexposed microorganisms in the contaminated microcosms that was provided by water renewal during the experiment.

Isoproturon (IPU)

Short-Term Effects

Gustavson et al. (2003) measured periphyton photosynthesis following exposure durations of 1 and 24 h, and found no effect concentration (NEC) values of 1 μg/L and 0.019 μg/L IPU, respectively. IPU was more toxic than the three other herbicides they tested (i.e., metribuzin, hexazinone, and pendimethalin).

Chronic Effects, PICT Assessment and Recovery Processes

Schmitt-Jansen and Altenburger (2005a, b, 2008) performed a series of investigations using 20 L glass aquaria to evaluate the effects on periphytic communities of a 14- or 28-day exposure to IPU (2.4–312 μg/L). Periphyton chl *a* fluorescence was inhibited at IPU concentrations above 20 μg/L, while algal populations shifted from diatoms to chlorophytes at concentrations in the range 20–312 μg/L. At the highest test concentrations, *Navicula halophila* was predominant in the diatom community (89%), but microscopy revealed abnormally shaped cells among these organisms. The replacement of diatom species was associated with an increase in tolerance, as was observed in short-term inhibition tests on photosynthesis (using pulse-amplitude-modulated (PAM) fluorometry or ^{14}C-carbonate incorporation). The authors also addressed their difficulty in comparing their observed effects with SSD predictions, because the database used for IPU toxicity on algae was very poor (Schmitt-Jansen and Altenburger 2005a).

In other studies, it was showed that diatom density and composition in periphyton were impacted by IPU at concentrations ranging from 5 to 30 μg/L; in addition, IPU treatments seemed to favor facultative heterotroph diatoms, which are able to switch trophic mode from autotrophy to heterotrophy (Pérès et al. 1996; Debenest et al. 2009). However, Laviale et al. (2010) observed that light, which can be considered as a direct physical stressor, slightly modulated the acute toxic effects of IPU on the photosynthesis of natural periphytic communities.

Knauert et al. (2009) also reported an impact of IPU on density and diversity of phytoplankton communities that were exposed for 5 weeks to a constant nominal IPU concentration of 14 μg/L. The observed effects followed similar patterns to those of diuron in the same study (see Sect. 2.1.2.1), and were followed by a recovery period as IPU dissipated from the water ($t_{1/2}$=35 days). In contrast to the results described above, Traunspurger et al. (1996) did not find any effects of IPU on phytoplankton cell abundance or community composition at nominal concentrations up to 90 μg/L, following an 8-week exposure. However, it is important to note that real IPU concentrations in the microcosms were not monitored, and microcosms were not replicated during this study.

Linuron

Chronic Direct Effects

The chronic effects of linuron on phytoplankton and periphtyon have been investigated using microcosms at concentrations ranging from 0.5 μg/L (Van Geest et al.

1999) to 500 μg/L (Daam et al. 2009a). Except for Van Geest et al. (1999), who found that three repeated linuron treatments (0.5–50 μg/L at 4-week intervals) caused only negligible changes in algal communities; all studies produced direct and indirect effects on phytoplankton and periphyton (Van den Brink et al. 1997; Slijkerman et al. 2005; Daam et al. 2007, 2009a). In tropical freshwater microcosms, linuron (15–500 μg/L) inhibited photosynthesis and affected both phytoplankton and periphytic communities (Daam et al. 2009a). The most sensitive species were chlorophytes belonging to the genera *Scenedesmus*, *Coelastrum*, and *Pediastrum* (phytoplankton) and the cyanobacterium *Chamaesiphon* sp. (periphyton). However, given the development of tolerant taxa (mainly belonging to diatom and cryptophyte classes) and functional redundancy, the authors emphasized that chl *a* concentration was not a sensitive indicator of linuron exposure, especially for the effects on phytoplankton. Daam et al. (2009b) compared the effects of linuron, in a microcosm study carried out in Thailand, with the effects reported in temperate model ecosystem studies. The authors concluded that the sensitivity of primary producers to the effects of linuron was similar among different climatic regions. Such similarity supports the use of toxicity data in tropical regions that were generated in temperate ones.

Chronic Indirect Effects

Van den Brink et al. (1997), using macrophyte dominated microcosms, demonstrated that linuron can also indirectly stimulate more tolerant phytoplankton species such as *Chlamydomonas sp*. Linuron exposure decreased macrophyte biomass, thereby increasing nitrate levels, which, in turn, produced an increase in total phytoplankton chl *a* levels. Such ecological cascading effects were confirmed by Slijkerman et al. (2005) and Daam and Van den Brink (2007), who showed that linuron-induced primary inhibition of the photosynthetic efficiency of primary producers (including macrophytes) resulted in a significant release of nutrients in the water, which consequently stimulated less-sensitive or fast-adapting phytoplankton species. Van den Brink et al. (1997) and Slijkerman et al. (2005) recorded an increase in flagellates subjected to a linuron treatment regime, whereas Daam and Van den Brink (2007) identified the algal genera *Ephitema*, *Navicula*, and *Closterium* as being favored by changes resulting from linuron exposure.

2.1.3 Chloroacetamides

Alachlor and Metolachlor

The effects of alachlor on periphytic (Spawn et al. 1997) and epipelic (Carder and Hoagland 1998) algal communities have been investigated in stream microcosms. Carder and Hoagland (1998) recorded a decrease in algal biovolumes after a 4-week exposure to 90 μg/L of alachlor, but no effects were detected at 5 μg/L. The relative abundance of the diatoms *Navicula* sp. and *Gyrosigma exinium* significantly

decreased following alachlor exposure, but effects persisted only for *G. exinium* at the highest concentration. Similarly, Spawn et al. (1997) did not observe any significant alachlor effect within 3 weeks of exposure at 1 μg/L, although algal biomass and cell densities were inhibited at all other concentrations (i.e., 10, 30, 100, and 1,000 μg/L). There was a shift in the dominant algae at concentrations of 30 μg/L and higher. The centric diatom *Melosira varians* was the most affected by alachlor, but other centric diatoms were not affected, demonstrating that similar taxa may exhibit different responses to this herbicide. By contrast, Debenest et al. (2009) showed that 30 μg/L of the chloroacetamide herbicide s-metolachlor had no effect on the abundance of *M. varians,* following a 3-day exposure. However, other diatom species such as *Eolimna minima* and *Navicula reichardtiana* proved sensitive to this herbicide, and a significant decrease in chl *c* concentrations and live-cell density was recorded in periphtyon exposed for 3 days to levels of 5 and 30 μg/L. Using complex outdoor microcosms, Relyea (2009) observed no significant effect of metolachlor (7.4 μg/L) on phytoplankton chl *a* or periphyton biomass within 16 or 35 days of exposure.

Other Chloroacetamides

Mohr et al. (2008b) studied the effects of metazachlor on plankton communities in pond and stream mesocosms over a monitoring period of 140 days. Metazachlor strongly affected both pond and stream communities at concentrations higher than 5 μg/L (i.e., 20–500 μg/L). Direct negative effects were most prominent for chlorophytes, whereas diatoms and cryptophytes seemed insensitive. Moreover, the herbicide remained highly persistent in the mesocosms ($t_{1/2}$ = 27–48 days), and chlorophytes did not recover in the more strongly contaminated stream mesocosms, suggesting potential long-lasting effects of metazachlor on phytoplankton in exposed aquatic ecosystems. This contrasted with the results of Noack et al. (2003), who found only slight effects of metazachlor on phytoplankton densities at very high concentrations (10,000 μg/L), followed by a recovery after 30–35 days. However, these authors advised that their conclusions be accepted with caution, because lack of replicated mesocosms prevented statistical evaluation of results. In model streams, the only effects recorded by Takahashi et al. (2007) on periphyton exposed to pretilachlor (26–382 μg/L) for 7–28 days were a slight increase of *Navicula pupula* and a slight decrease of *Anabaena sp*. Using complex outdoor microcosms, Relyea (2009) observed a decrease in phytoplankton chl *a* after a 16-day exposure to 10 μg/L acetochlor, whereas periphyton biomass remained unaffected throughout the entire duration of the study (35 days).

2.1.4 Sulfonylureas

Using four levels of the sulfonylurea herbicide metsulfuron-methyl (0, 1, 5, and 20 μg/L) in freshwater enclosures, Wendt-Rasch et al. (2003) showed an increase in the biomass of periphytic algae growing on the leaves of the macrophyte *Myriophyllum spicatum,* after a 2-week exposure. They attributed this increase to a

possible nutrient leakage from the macrophyte leaves following herbicide exposure. Changes were also detected on the species composition of periphytic algal communities in enclosures exposed to 20 μg/L, while metsulfuron-methyl did not alter phytoplankton community biomass or composition. This suggests that the toxicity of sulfonylurea herbicides on phytoplankton is limited, as asserted by Seguin et al. (2001) and Leboulanger et al. (2001), who showed that nicosulfuron was far less toxic to phytoplankton than was atrazine. Nevertheless, they emphasized that nicosulfuron (10 μg/L) can affect phytoplankton species composition by inhibiting more diatoms than chlorophytes. Abdel-Hamid et al. (1996) also observed community composition effects on lake phytoplankton from exposure (1, 10, and 100 μg/L) to another sulfonylurea herbicide, chlorosulfuron.

2.1.5 Glyphosate

Effects of High Glyphosate Concentrations

Vera et al. (2010) showed that a high concentration of the commercial formulation of Roundup® (8 mg/L of the active molecule glyphosate) produced a clear delay in periphytic colonization and reduced periphytic dry weight, as well as chl *a*, in comparison with control mesocosms. This occurred despite a significant increase in total phosphorus concentrations in the treated mesocosms. Pérez et al. (2007) also observed a significant phosphorus release in mesocosms after the addition of Roundup® (6 and 12 mg/L of the active ingredient glyphosate), which was associated with structural changes in the planktonic and periphytic microbial assemblages, within a few days. In treated mesocosms, total phytoplankton abundance decreased, whereas primary production and picocyanobacteria abundance increased. Similar patterns have been observed in periphyton, which showed increasing abundance of cyanobacteria following glyphosate exposure (Vera et al. 2010). Schaffer and Sebetich (2004) also found that Rodeo® treatments (0.125 and 12.5 mg/L of the active ingredient glyphosate) led to significant stimulation of primary productivity of a lake phytoplankton community during a 7-h incubation period. They hypothesized that this effect could have resulted from the use of the nitrogen and phosphorus released through the glyphosate degradation process. Similarly, Relyea (2005) observed an increase in periphytic algal biomass after a 2-week Roundup® exposure (3.8 mg glyphosate/L), which, he suggested, could result from a decrease in grazing pressure caused by Roundup® effects on herbivorous organisms.

Chronic Effects of Environmentally Relevant Glyphosate Concentrations

Using a more environmentally relevant glyphosate concentration (i.e., 6.9 μg/L), Relyea (2009) showed that Roundup® caused insignificant effects on an aquatic food web comprising periphyton, phytoplankton, and other higher trophic level organisms. This was consistent with results from Pesce et al. (2009), who demonstrated that any effects of glyphosate (10 μg/L; 14 days) on riverine algal communities was limited to ones of community composition (assessed by microscopic

identification and PCR-DGGE on 18S rDNA gene fragment). Moreover, effects were only perceptible on communities sampled during the summer, whereas spring communities remained insensitive, revealing that response of natural algae to herbicide exposure can be seasonally dependent. Glyphosate-induced effects (at 1, 10, and 100 μg/L) on phytoplankton diversity were also reported by Abdel-Hamid et al. (1996) in a 13-day outdoor enclosure experiment.

2.1.6 2,4-Dichlorophenoxyacetic Acid (2,4-D)

Using bioassays on mesotrophic lake phytoplankton communities exposed to different herbicides, Brown and Lean (1995) showed that 2,4-D exhibited the least toxic effect on photosynthetic activity and phosphate and ammonium uptake rates. This herbicide had EC_{50} values (3 h) higher than 33 mg/L for these three biological end points. Using an 8-day microcosm study, Kobraei and White (1996) observed a stimulation of primary production in phytoplankton communities exposed to 2 mg/L of 2,4-D. No stimulation was detected at 10 mg/L, yet higher concentrations negatively affected phytoplankton. Gross primary production, community respiration, chl *a* concentrations, algal density and biovolumes significantly decreased in the 100 and 1,000 mg/L microcosms. They found heterotrophic algal taxa to be the least affected by 2,4-D at highest concentrations. Using lower concentrations in large outdoor mesocosms, Relyea (2005, 2009) showed that levels of neither 16 μg/L nor 120 μg/L of 2,4-D produced significant direct or indirect effects on periphyton and phytoplankton biomass.

2.1.7 Other Herbicides

Chronic Effects on Biomass and Primary Production

Testing the effects of ten herbicides commonly applied in rice cultivation (clomazone, thiobencarb, pendimethalin, propanil, quinclorac, halosulfuron, bensulfuron-methyl, triclopyr, 2,4-D-amine, and molinate) in aquaculture ponds at rates equivalent to direct application, Perschbacher et al. (2002) found that, with the exception of propanil, none of the herbicides had any measurable effect on phytoplankton primary production or chl *a* levels, within 9 exposure days. Propanil stimulated chl *a* (Perschbacher et al. 1997, 2002). A 3-day microcosm study by Waiser and Robarts (1997) showed that the carbamate herbicide triallate exhibited limited effects on lake phytoplankton biomass, since substantial declines in chl *a* concentrations only occurred at a triallate concentration of 1 mg/L (but not 10 and 100 μg/L).

Chronic Effects on Community Composition

Caquet et al. (2005) investigated the effects of the herbicide fomesafen (40 μg/L), alone and in combination with an adjuvant (Agral 90, 90 μg/L), on plankton

communities in outdoor mesocosms over a 9-month period. They found that Agral 90 did not influence the effect of fomesafen on phytoplankton. Fomesafen inhibited Chlorophyceae but produced abundance and biovolume increases of Cyanobacteria, Cryptophycea, Dinophycea, and Bacillariophycea, thus enhancing taxonomic phytoplankton diversity. Phytoplankton community composition was also affected by the herbicide methabenzthiazuron, during a 5-month microcosm study (Wellmann et al. 1998). Population dynamics were dependent on herbicide concentrations as well as light intensity, temperature, and nutrient concentrations. Primary production was temporarily inhibited at metabenzthiazuron concentrations ranging from 89 to 3,371 μg/L, while lower concentrations (10, 21 and 43 μg/L) induced no or only transient weak responses in the phytoplankton. The most sensitive algae belonged to Chlorophyceae, whereas Cryptophycea exhibited strong recovery at the higher concentrations during the study period. In field-paddy mesocosms, Sánchez et al. (2006) observed no significant effect of the rice herbicide profoxydim (at rates from 45 to 377 g/ha) on phytoplankton density and composition, within 9 exposure days. Using in situ enclosures, Faber et al. (1998) tested the effect of the herbicides glufosinate-ammonium and bialaphos on phytoplankton communities in a eutrophic lake. At the highest treatment levels (10 mg/L), both herbicides caused a significant decrease in small phytoplankton cell species (1–3 μm). The effects were transient, and recovery was observed earlier for bialaphos (14 days post application) than for glufosinate-ammonium (49 day). Larger phytoplankton was generally not adversely impacted by these herbicides.

2.2 Effects of Herbicide Mixtures

2.2.1 Mixtures of Similarly Acting Herbicides

The effects of mixtures of PS-II inhibitors have been investigated on freshwater microbial communities in several studies; the tested mixtures were of various triazine (Pollehne et al. 1999) and/or phenylurea herbicides (Knauert et al. 2008, 2009; Knauer et al. 2010, Pesce et al. 2010b). Using a 10-day mesocosm approach, Pollehne et al. (1999) found no herbicide-specific effects on estuarine phytoplankton communities exposed to the combined triazines simazine and atrazine, at concentrations of 0.04–6 μg/L each. Knauert et al. (2008, 2009) performed a 5-week outdoor mesocosm study to evaluate the effects of a mixture of equitoxic concentrations of atrazine, isoproturon and diuron on phytoplankton photosynthesis and community succession. The herbicide mixture adversely affected photosynthetic activity and significantly influenced community structure in terms of abundance, diversity, and species composition. The results demonstrated that the combined effects of the three PS-II inhibitors herbicides could be predicted, based on the concept of concentration addition (Faust et al. 1994). This outcome was in line with previous findings from phytoplankton bioassays, in which the combined effects of similarly acting herbicides were assessed (e.g., Faust et al. 2001; Junghans et al. 2003; Chèvre

et al. 2006). Similarly, Pesce et al. (2010b), using a PICT approach and photosynthesis bioassays, demonstrated that mixtures of diuron and its metabolite N-(3,4-dichlorophenyl)-*N*-methylurea (DCPMU) produced additive effects on natural phototrophic biofilm photosynthesis.

Knauer et al. (2010) showed that long-term exposure to PS-II inhibitor mixtures can also induce cotolerance within phytoplankton communities. However, they also demonstrated that even if cotolerance is expected for compounds having similar biochemical modes of action, this cotolerance may vary among molecules (in their study, atrazine, isoproturon, and diuron).

2.2.2 Mixtures of Dissimilarly Acting Herbicides

Carder and Hoagland (1998) and Hartgers et al. (1998) assessed the effects of mixtures of PS-II inhibitors (triazine and/or phenylurea) and the chloroacetanilides, which are known to affect fatty acid metabolism (Couderchet et al. 1998). The effects of a combination of atrazine (12 and 150 μg/L) and alachlor (5 and 90 μg/L) on benthic algal communities in artificial streams appeared to be additive rather than synergistic and led to a significant decrease in cell biovolumes throughout the 4-week experiment (Carder and Hoagland 1998). Hartgers et al. (1998) assessed the response of phytoplankton communities to a mixture of atrazine, diuron, and metolachlor in 28-day freshwater microcosms (600 L); concentrations were 0.01–1.0-fold the EC_{50} (72 h) values. These EC_{50} values were obtained in standard algal tests using *Selenastrum capricornutum* and were performed to comply with OECD guidelines. Direct effects were detected at 0.3-fold the EC_{50} treatment level and higher. Effects included a drop in photosynthetic efficiency and a decrease in the abundance of several phytoplankton taxa, especially the cholorophyceae *Monoraphidium* sp., whereas other species such as *Cyclotella* or *Chlamydomonas* sp. showed a marked increase in abundance as doses increased. However, it was not possible for the authors to determine how the three tested compounds interacted. Relyea (2009) tested a mixture of low levels (2–16 μg/L) of five herbicides (atrazine, acetolachlor, metolachlor, glyphosate, and 2,4-D) in a 36-day mesocosm experiment; results showed that phytoplankton chl *a* effects occurred, but, except for acetochlor, differed from those of metolachlor, glyphosate, 2,4-D, and atrazine alone. The five-herbicide mixture had similar effects on periphyton abundance as did exposure to each of the five herbicides alone.

2.2.3 Mixtures of Herbicides with Other Organic Pesticides

Herbicides and Insecticides

Wendt-Rasch et al. (2003) investigated the effects of the sulfonylurea herbicide metsulfuron-methyl (1, 5 and 20 μg/L) alone and in combination with the pyrethroid

insecticide cypermethrin (0.05 μg/L) over a 2-week period, in freshwater enclosures. They recorded no combined direct or indirect effects of the two compounds on periphyton and phytoplankton communities. Van den Brink et al. (2009) evaluated the chronic (8 week) effects in microcosms of a mixture of the triazine herbicide atrazine and the organochlorine insecticide lindane at five equivalent concentrations, ranging from 0.01 to 5 times the EC_{50} of the most sensitive standard test organism (*Scenedesmus subspicatum* for atrazine and *Oncorhynchus mykiss* for lindane). Results were that phytoplankton chl *a* increased, following an increase in *Cyclotella* species, at the highest treatment rate during weeks 5 and 6. The authors suggested that the effects of atrazine on phytoplankton were lower than expected and were counteracted by reduced grazing pressure from lindane-induced effects on zooplankton. Effects on periphyton were only detectable at the species level. At the highest treatment level, the effects produced were characterized as increased population density of the chlorophyceae genus *Characium,* and decreased population densities of the diatom genus *Achnanthes* and the cyanobacterium *Oscillatoria redeckei*. This result suggests that a cause–effect relationship existed at the highest treatment level. The authors hypothesized that the pesticide mixture affected both top-down and bottom-up regulation mechanisms.

Herbicides and Fungicides

Villeneuve et al. (2011) and Tlili et al. (2011) investigated the responses of periphytic communities to pesticide mixture exposures of the herbicide diuron and the fungicides azoxystrobin or tebuconazole, respectively. However, the main objective of these two studies was not to examine the interactions between the tested compounds. Rather, it was to assess the influence of flow regimes (Villeneuve et al. 2011) or compare chronic versus acute exposure effects (Tlili et al. 2011), using pesticide mixtures and concentrations classically encountered in a vineyard watershed. Therefore, even if the direct effects of diuron on periphytic communities were clearly detectable in the two studies, it was not possible to evaluate if simultaneous exposure to a fungicide modulated the response of the impacted communities.

2.2.4 Successive Treatments

One strategy to assess the fate and ecological effects of agricultural pesticide treatments is to simulate the events that often transpire to contaminate surface waters during actual pesticide application programs. The simulation procedure consists of emulating real agricultural application scenarios by making successive treatments with various pesticides to study the effects of residues leached into aquatic ecosystems. By employing ditch mesocosm studies over a period of 30 weeks, Arts et al. (2006) tested the effects of 15 separate spray treatments to potatoes with various compounds (prosulfocarb, metribuzin, lambda-cyhalothrin, chlorothalonil,

fluazinam) at 0.2, 1, and 5% of the respective recommended application rates. Most effects were observed at the 5% treatment level, which resulted in short-term changes to pH and oxygen levels; phytoplankton responded in a manner that was consistent with expected compound-specific results. These study results showed that the successive impact of repeated treatments by the various pesticides did not produce extensive harm, since most substances dissipated rapidly, avoiding simultaneous exposure for most combinations. In a similar experiment, van Wijngaarden et al. (2004) mimicked an application scenario in tulip-cultivation practice. They made successive treatments of the fungicide fluazinam, the insecticide lambda-cyhalothrin and the herbicides asulam and metamitron to indoor microcosms at estimated spray-drift concentrations varying from 0.2 to 5% of recommended label rates. The 0.5% treatment regime resulted in short-term effects, whereas the 2 and 5% treatment levels triggered marked effects. Although effects were detected at the ecosystem level, the two highest herbicide application levels had only minimal effects on phytoplankton and periphyton. Phytoplankton biomass increased from indirect effects; these effects resulted from the decrease of the macrophyte *E. nuttallii* after asulam application (decreased competition for nutrients), and from the decrease of zooplankton after lambda-cyhalothrin application (reduced grazing pressure). Treatments had no direct or indirect effects on the abundance of periphyton. Nevertheless, using the same pesticide application procedure, Wendt-Rasch et al. (2004) showed that the final effect of pesticide exposure was greatly influenced by the structure of the ecosystems. In mesotrophic microcosms, dominated by submerged macrophytes, periphyton biomass increased and species composition varied at the 0.5% treatment level and higher. However, there was no effect on these two parameters in eutrophic microcosms, characterized by a high *Lemna* surface coverage.

3 Field Studies

3.1 Effect of In Situ Exposure on Community Structure and Primary Production

Lotic ecosystems, especially in agricultural areas, are often highly exposed to herbicide pollution. A common way to assess the resulting effects on aquatic communities is to compare biological parameters at different sampling sites that received different herbicide levels (ideally including a clean reference point). This strategy was successfully used by Dorigo et al. (2002) to measure changes to phytobenthic community species composition in river sections, mainly contaminated by atrazine and isoproturon. Using partial 18S rRNA cloning and sequencing, they showed that the proportion of diatoms was lower at the unpolluted site than at polluted ones. In an agricultural area, Pesce et al. (2008) observed a sharp drop in free algal biomass during the main pollution period, which suggested a strong herbicide impact on phototrophic

communities. However, Dorigo et al. (2002) and Pesce et al. (2008) urged caution in interpreting these results, because of the complexity in distinguishing between effects of pollutants vs. other environmental variables. Morin et al. (2009) have also recently stressed the difficulties in accurately linking diatom community structure to pesticide inputs in lotic environments. However, Ricart et al. (2010) revealed a potential relationship between triazine-type herbicides and diatom community distribution in a contaminated river (Llobregat river, Spain). Their study also showed that the metrics most sensitive to the presence of pesticides were chl *a* and photosynthetic activity.

A series of in situ studies have recently been conducted in a small river that drains a vineyard watershed (Morcille River, France; Montuelle et al. 2010). Various surveys revealed that biofilm phototrophic community composition varied from upstream to downstream locations, in parallel with increased nutrient and pesticide concentrations. Changes in phototrophic community composition along this river have been recorded using several end points: pigment distribution, eukaryotic gene structure, and diatom taxonomic composition (Dorigo et al. 2007, 2009, 2010a, b; Morin et al. 2010; Pesce et al. 2010a, b).

3.2 PICT Approaches

Among the various methods and tools available to evaluate microbial community responses to toxicant exposure, the PICT approach makes it possible to partially isolate the effects of individual toxicants within an ecosystem that is subjected to multiple stressors, by studying shifts in community sensitivity (Schmitt-Jansen et al. 2008). In the river Morcille, PICT was, therefore, applied to verify that structural changes in phototrophic communities were related to pesticide contamination. In all surveys, all biofilms exhibited an upstream-to-downstream increase in tolerance to diuron, which is the most often detected herbicide in this river (Dorigo et al. 2007, 2009, 2010a, b; Pesce et al. 2010a, b). Pesce et al. (2010a) identified three possible influences that constituted covarying environmental variables (i.e., nitrates, conductivity, and temperature) in the tolerance induction. However, statistical analysis demonstrated that the main factor affecting diuron sensitivity was the mean in situ diuron exposure level during the biofilm colonization periods.

Nevertheless, field studies conducted by Guasch et al. (1998a, b, 2003) in various European streams and rivers clearly indicated that the sensitivity of phototrophic biofilms to organic herbicides in lotic systems may be highly dependent on light conditions during colonization. Indeed, they observed higher atrazine toxicity to natural biofilms that were adapted to high-light conditions, and were dominated by green algae or cyanobacteria, than that to diatom-dominated biofilms adapted to low-light conditions.

Dorigo et al. (2004) assessed seasonal changes in the sensitivity of river microalgae to atrazine and isoproturon along a contamination gradient, and showed that both free and fixed algal communities responded positively to the PICT approach. The positive response occurred despite the fact that free algae are mobile and can

reduce their exposure time to toxicants by escaping from adverse situations. Both periphyton and phytoplankton can also be used to run PICT approaches in lake ecosystems, as shown by Nyström et al. (2002) and Bérard et al. (2003), who assessed the toxic effects of Irgarol 1051 and/or atrazine on microalgal communities in Lake Geneva

3.3 Recovery Studies

In only a few, more recently performed studies have researchers examined recovery processes of phototrophic communities, following herbicide exposure under natural river contamination conditions (Dorigo et al. 2010a, b; Morin et al. 2010; Rotter et al. 2011). The authors of these four studies have attempted to characterize the dynamics of recovery of periphytic communities transplanted from herbicide-contaminated sites to "nonpolluted" reference sites. The recovery processes were evaluated for changes in community structure (biomass, distribution of algal classes), diversity (diatom taxonomic composition, 18S PCR-DGGE band patterns) and tolerance capacities, using PICT-approaches for the most predominant herbicide in the studied rivers (i.e., diuron in Dorigo et al. 2010a, b and prometryn in Rotter et al. 2011). The results indicated a high recovery potential for periphytic communities. Evidence supported the view that communities recovered, at least partially, in structural, diversity, and functional attributes, after a few weeks within reference sites. Accordingly, Dorigo et al. (2010a, b) and Rotter et al. (2011) emphasized that the use of biofilm recovery capacity could potentially be a suitable management tool for analysis of recovery processes in freshwater ecosystems, especially when using the PICT-concept. However, Morin et al. (2010) emphasized that immigration and emigration of algal species certainly takes place in such transplantation experiments. Therefore, the observed trajectories of recovery were probably assisted by such species migration, rather than resulting only from the new exposure conditions at transplantation sites.

4 Potential Future Areas for Research

Among many future areas of research, three main promising ones have been identified:

- Improvement of exposure characterization, and improved measurement of bioavailable contaminant concentrations
- Improvement or diversification of effects characterization from individual to the community level
- Assessment of environmental restoration and ecological trajectories after removal of toxic pressure

4.1 Improving Exposure Assessments

4.1.1 The Question of Mixtures

Despite the fact that pesticides frequently occur in mixtures, our review clearly shows a dearth of studies that evaluate pesticide mixture effects on autotrophic microbial communities. Even fewer mixture studies have been performed under environmentally relevant conditions. This point has recently been emphasized by Van den Brink et al. (2009), who noted the scarcity of data on community-level effects of pesticide mixtures. Several authors have emphasized the need to consider mixtures, when assessing the ecological effects of pesticides (Chèvre et al. 2006; Knauer et al. 2010). However, there is still debate over the best way to address this issue (Knauert et al. 2008, 2009), and it can be argued that the assessment of mixture effects is in its infancy (Belden et al. 2007). DeLorenzo et al. (2001) emphasized the need to address the toxicity of pesticide degradation products to aquatic microorganisms. The integration into risk assessments of the effects shown by pesticide metabolites, both alone and in pesticide mixtures, would significantly improve ecological risk assessment processes (Sinclair and Boxall 2003). Unfortunately, there is still too little research of this type being performed. Another progressive step will be to take into account the mode of action of pesticides, since mechanistic insights may help explain the toxicity of mixtures (additivity or independence of action; Chèvre et al. 2006; Ricart 2011).

4.1.2 Benefiting from the Development of Chemical Tools

Another area in which progress is needed is development of better chemical sensing and field sampling devices. The recent development of passive sampling techniques for monitoring organic pesticides in freshwaters (e.g., polar organic chemical integrative samplers, diffusive gradients in thin films, semipermeable membrane devices, silicon rods, etc.) opens new avenues to screen for a large variety of organic and inorganic contaminants. Such improvements would facilitate the assessment of the relationship between community-level tolerance induction and mean contaminant exposure (Pesce et al. 2010b; Rotter et al. 2011). Moreover, recently, some authors have proposed combining passive samplers with bioassays to assess the toxicity of toxicant mixtures extracted directly from the environment. This combination method may constitute a simple and cost-effective way to determine potential acute effects of contaminant mixtures in various aquatic environments (e.g., Muller et al. 2007; Liscio et al. 2009; Shaw et al. 2009). Recently, polar organic samplers have been combined with photosynthesis bioassays (using microalgae cultures) to assess phytotoxicity of various mixtures of organic toxicants (Escher et al. 2006; Muller et al. 2007; Shaw et al. 2009). The use of natural phototrophic microbial communities in such an approach could improve outcomes and usefulness of previous results, which combined

polar organic samplers with monospecific photosynthesis bioassays (Pesce et al. 2011).

4.1.3 Exposure Characteristics and Dynamics (Chronic-Acute)

The question whether there is a relevant exposure measure for periphytic microorganisms that are embedded in an ExoPolySaccharide (EPS) matrix is open: depending on the solubility of pesticides, the concentrations in the water phase may not be the most useful for predicting biological effects. Rather, periphytic assemblage studies should address exposure, by focusing on biofilm-adsorbed pesticide concentrations, especially for hydrophobic compounds. Indeed, the sorption of pesticides on periphyton can enhance toxicity by extending exposure time (Dorigo et al. 2010a). Pesticide sorption also drives bioaccumulation processes by favoring pesticide transfer to higher trophic levels *via* periphyton consumers, such as grazers.

Exposures are naturally dynamic and comparing the consequences of long-term low-dose (i.e., chronic exposure) vs. short-term high-dose (i.e., acute exposure) exposures is very difficult to accomplish. Recent chemical monitoring studies have shown that, during floods, many pollutant fluxes – including those of pesticides – can vary over several orders of magnitude, especially in small stream systems (Rabiet et al. 2009). In small stream ecosystems, environmental exposure of aquatic communities to pesticides can rapidly increase during rainfall events. Therefore, special attention should be given to (a) studying pulsed-exposures that result from episodic runoff events and (b) addressing the ecotoxicological question of how to predict the lethal and sublethal consequences of such population exposures (Tlili et al. 2008, 2011). Furthermore, the effect of long-term, low-dose pesticide exposures may produce effects (e.g., biodiversity changes, tolerance acquisition, and functional changes) that only become apparent in organisms after several generations.

4.2 Improving Assessment of Biological Effects

4.2.1 From Monospecific Tests to Community Assessment

There have long been efforts to enhance the integration of ecology and ecotoxicology. However, it is now well established that a more suitable model than single-species testing is to assess the ecological effects of pesticides at the microbial community level. Nevertheless, data from monospecific bioassays will also be required. Hence, the SSD approach has become a practical ecological risk assessment method and decision-making processes to determine water quality criteria (Schmitt-Jansen and Altenburger 2005b). Although SSD approaches are useful in environmental risk research (Schmitt-Jansen et al. 2008), especially if toxicity datasets are sufficiently

robust (Schmitt-Jansen and Altenburger 2005a), they cannot fully replace model ecosystems (microcosms, mesocosms, or enclosures) or field investigations. The reason is that SSD approaches focus on short-term or midterm effects and tend to ignore important ecological factors (Brock et al. 2004), such as the indirect effects that result from community interactions (i.e., interactions among zooplankton, benthic grazers, heterotrophic microbial communities, etc.).

4.2.2 Using Molecular Tools in Ecotoxicology

Although molecular biology has revolutionized the understanding of microbial ecology in various environments, including water ecosystems, its use in ecotoxicology has probably been underused, although we did find a few studies that employed molecular techniques such as PCR-DGGE (e.g., Dorigo et al. 2007; Tlili et al. 2008; Pesce et al. 2009) or 18S rRNA cloning and sequencing (Dorigo et al. 2002). New sets of 18S rRNA primers that are more specific to different taxonomic levels (e.g., Chlorophycea and Bacillariophyceae; Valiente Moro et al. 2009) could prove to be highly valuable for studying the phototrophic community dynamics, after pesticide exposure. Cutting-edge molecular tools such as (meta)genomics or microarrays also offer new and powerful possibilities for assessing pesticide effects on microbial community (including phototrophic communities) diversity and functionality.

4.2.3 Understanding the Ecological Consequences of Tolerance Acquisition

For many research scientists the only realistic approach to obtain aquatic ecotoxicology data consists of performing in situ studies (Boudou and Ribeyre 1997). Field studies, however, may yield more useful results, although distinguishing between pollutant effects and those related to other physical, chemical or biological environmental variables can be very challenging. As mentioned above (Sect. 3), PICT is one of the tools best adapted to achieve this goal because tolerance to one toxicant is less sensitive than is other community characteristics to natural variations at sampling sites (Schmitt-Jansen et al. 2008). To improve PICT methodology, special attention should be paid to cotolerance patterns and to developing new short-term tests designed to evaluate tolerance capacities, especially with a view to broadening the range of toxicants monitored (Blanck 2002; Tlili and Montuelle 2011).

4.3 Ecosystem Recovery

Finally, interest in restoring chemically polluted ecosystems is growing, especially through environmental policies such as the European Water Framework Directive (EU 2000). In this Directive, the EU commits its members to achieve good

qualitative and quantitative ecological status of all surface waters, and there is now growing interest in studying recovery trajectories. Ecosystem recovery is defined as the potential for a disturbed ecosystem to return to a state similar to that before a stress was imposed on it, which basically revolves around the notion of ecosystem and community resilience. Despite the importance of studying and understanding the resilience processes employed by autotrophic microbial communities following pesticide exposure, even basic knowledge on these processes remains scarce (e.g., Morin et al. 2010; Dorigo et al. 2010a, b; Rotter et al. 2011).

5 Summary

Over the past 15 years, significant research efforts have been channeled into assessing the effects of organic herbicides on freshwater phototrophic microbial communities. The results of this research are reviewed herein. The main conclusions we have reached after performing this review can be summarized into five points:

- Most relevant assessments have dealt with the effects of triazine and phenylurea herbicides. Herbicides from these chemical classes are often considered to be model compounds when photosystem-II inhibitors are studied.
- Until the early 2000s, the vast majority of investigations conducted to evaluate herbicide effects on phototropic microbes were performed in microcosms or mesocosms. In such studies, herbicides were usually applied alone, and often at concentrations much higher than those detected in the environment. More recently, the trend has been toward more realistic and relevant studies, in which lower herbicide concentrations were considered, and compound mixtures or successive treatments were tested. Increasingly, in situ studies are being designed to directly evaluate microbial community responses, following chemical exposures in contaminated aquatic environments.
- Several biological end points are used to evaluate how organisms in the phototrophic microbial community respond to herbicide exposure. These end points allow the detection of quantitative changes, such as chl *a* concentrations, total cell counts or periphytic biomass, qualitative changes such as community structure to algal diversity, or functional changes such as photosynthesis and respiration, among others. They may give different and complementary information concerning the responses of microbial communities.
- PICT approaches, which have generally combined functional and structural measurements, may prove to be valuable for assessing both an immediate impact, and for factoring in the contamination history of an ecosystem at the community level.
- Finally, any relevant assessment of pesticide effects should incorporate a detailed environmental characterization that would include abiotic parameters (light, flow speed, nutrient content), or biotic parameters (diversity and structure of biofilms), because these control the bioavailability of pesticides, and thereby the exposure of microbial communities.

To improve the value of ecotoxicological risk assessments, future research is needed in two key areas: first, more information on the effects of pollutants at the community level must be obtained (new tools and new end points), and second, more effort must be directed to reinforce the ecological relevance of toxicological investigations.

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References

Abdel-Hamid MI, Källqvist T, Hessen DO, Berge D (1996) The use of field enclosure experiments to study the effect of pesticides on lake phytoplankton. Lakes Reserv Res Manag 2: 199–209.

Arts GHP, Buijse-Bogdan LL, Belgers JDM, van Rhenen-Kersten CH, van Wijngaarden RPA, Roessink I, Maund SJ, van den Brink PJ, Brock TCM (2006) Ecological impact in ditch mesocosms of simulated spray drift from a crop protection program for potatoes. Integr Environ Assess Manag 2: 105–125.

Belden JB, Gilliom RJ, Lydy MJ (2007). How well can we predict the toxicity of pesticide mixtures to aquatic life? Integr Environ Assess Manag 3: 364–372.

Bérard A, Leboulanger C, Pelte T (1999a) Tolerance of *Oscillatoria limnetica* Lemmermann to atrazine in natural phytoplankton populations and in pure culture: influence of season and temperature. Arch Environ Contam Toxicol 37: 472–479.

Bérard A, Pelte T, Druart JC (1999b) Seasonal variations in the sensitivity of Lake Geneva phytoplankton community structure to atrazine. Arch Hydrobiol 145: 277–295.

Bérard A, Benninghoff C (2001) Pollution-induced community tolerance (PICT) and seasonal variations in the sensitivity of phytoplankton to atrazine in nanocosms. Chemosphere 45: 427–437.

Bérard A, Dorigo U, Mercier I, Becker-van Slooten K, Grandjean D, Leboulanger C (2003) Comparison of the ecotoxicological impact of the triazines Irgarol 1051 and atrazine on microalgal cultures and natural microalgal communities in Lake Geneva. Chemosphere 53: 935–944.

Blanck H (2002) A critical review of procedures and approaches used for assessing pollution-induced community tolerance (PICT) in biotic communities. Hum Ecol Risk Assess 8: 1003–1034.

Boudou A, Ribeyre F (1997) Aquatic ecotoxicology: From the ecosystem to the cellular and molecular levels. Environ Health Persp 105: 21–35.

Brock TCM, Crum SJH, Deneer JW, Heimbach F, Roijackers RMM, Sinkeldam JA (2004) Comparing aquatic risk assessment methods for the photosynthesis-inhibiting herbicides metribuzin and metamitron. Environ Pollut 130: 403–426.

Brown LS, Lean DRS (1995) Toxicity of selected pesticides to lake phytoplankton measured using photosynthetic inhibition compared to maximal uptake rates of phosphate and ammonium. Environ Toxicol Chem 14: 93–98.

Caquet T, Deydier-Stephan L, Lacroix G, Le Rouzic B, Lescher-Moutoué F (2005) Effects of fomesafen, alone and in combination with an adjuvant, on plankton communities in freshwater outdoor pond mesocosms. Environ Toxicol Chem 24: 1116–1124.

Carder JP, Hoagland KD (1998) Combined effects of alachlor and atrazine on benthic algal communities in artificial streams. Environ Toxicol Chem 17: 1415–1420.

Chang KH, Sakamoto M, Ha JY, Miyabara Y, Nakano SI, Doi H, Hanazato T (2011) Response of the plankton community to herbicide application (triazine herbicide, simetryn) in a eutrophicated system: short-term exposure experiment using microcosms. Limnology 12: 11–16.

Chapman PM (2002) Integrating toxicology and ecology: putting the "eco" into ecotoxicology. Mar Pollut Bull 44: 7–15.

Chèvre N, Loepfe C, Singer H, Stamm C, Fenner K, Escher BI (2006) Including mixtures in the determination of water quality criteria for herbicides in surface water. Environ Sci Technol 40: 426–435.

Clements WH, Rohr JR (2009) Community responses to contaminants: using basic ecological principles to predict ecotoxicological effects. Environ. Toxicol. Chem. 28: 1789–1800.

Couderchet M, Schmalfuss J, Böger P (1998) A specific and sensitive assay to quantify the herbicidal activity of chloroacetamides. Pestic Sci 52: 381–387.

Daam MA, Van den Brink PJ (2007) Effects of chlorpyrifos, carbendazim and linuron on the ecology of a small indoor aquatic microcosm. Arch Environ Contam Toxicol 53: 22–35.

Daam MA, Rodrigues AMF, Van den Brink PJ, Nogueira AJA (2009a) Ecological effects of the herbicide linuron in tropical freshwater microcosms. Ecotoxicol Environ Safe 72: 410–423.

Daam MA, Van den Brink PJ, Nogueira AJA (2009b) Comparison of fate and ecological effects of the herbicide linuron in freshwater model ecosystems between tropical and temperate regions. Ecotoxicol Environ Safe 72: 424–433.

Debenest T, Pinelli E, Coste M, Silvestre J, Mazzella N, Madigou C, Delmas F (2009) Sensitivity of freshwater periphytic diatoms to agricultural herbicides. Aquat Toxicol 93: 11–17.

DeLorenzo ME, Scott GI, Ross PE (1999) Effects of the agricultural pesticides atrazine, deeethylatrazine, endosulfan, and chlorpyrifos on an estuarine microbial food web. Environ Toxicol Chem 18: 2824–2835.

DeLorenzo ME, Scott GI, Ross PE (2001) Toxicity of pesticides to aquatic microorganisms: A review. Environ Toxicol Chem 20: 84–98.

Detenbeck NE, Hermanutz R, Allen K, Swift MC (1996) Fate and effects of the herbicide atrazine in flow-through wetland mesocosms. Environ Toxicol Chem 15: 937–946.

Devault DA, Merlina G, Lim P, Probst JL, Pinelli E (2007) Multi-residues analysis of pre-emergence herbicides in fluvial sediments: application to the mid-Garonne River. J Environ Monit 9: 1009–1017.

Dorigo U, Bérard A, Humbert JF (2002) Comparison of eukaryotic phytobenthic community composition in a polluted river by partial 18S rRNA gene cloning and sequencing. Microb Ecol 44: 372–380.

Dorigo U, Bourrain X, Bérard A, Leboulanger C (2004) Seasonal changes in the sensitivity of river microalgae to atrazine and isoproturon along a contamination gradient. Sci Total Environ 318: 101–114.

Dorigo U, Leboulanger C, Bérard A, Bouchez A, Humbert JF, Montuelle B (2007) Lotic biofilm community structure and pesticide tolerance along a contamination gradient in a vineyard area. Aquat Microb Ecol 50: 91–102.

Dorigo U, Lefranc M, Leboulanger C, Montuelle B, Humbert JF (2009) Spatial heterogeneity of periphytic microbial communities in a small pesticide-polluted river. FEMS Microbiol Ecol 67: 491–501.

Dorigo U, Bérard A, Rimet F, Bouchez A, Montuelle B (2010a) In situ assessment of periphyton recovery in a river contaminated by pesticides. Aquat Toxicol 98: 396–406.

Dorigo U, Bérard A, Bouchez A, Rimet F, Montuelle B (2010b) Transplantation of microbenthic algal assemblages to assess structural and functional recovery after diuron exposure. Arch Environ Contam Toxicol 59: 555–563.

Downing HF, DeLorenzo ME, Fulton MH, Scott GI, Madden CJ, Kucklick JR (2004) Effects of the agricultural pesticides atrazine, chlorothalonil, and endosulfan on South Florida microbial assemblages. Ecotoxicology 13: 245–260.

Escher BI, Quayle P, Muller R, Schreiber U, Mueller JF (2006) Passive sampling of herbicides combined with effect analysis in algae using a novel high-throughput phytotoxicity assay (Maxi-Imaging-PAM). J Environ Monit 8: 456–464.

EU (2000) Directive 2000/60/EC of the European parliament and of the council establishing a framework for Community action in the field of water policy. European Parliament, The Council, PE-CONS 3639/1/00 REV 1 EN.

Faber MJ, Thompson DG, Stephenson GR, Boermans HJ (1998) Impact of glufosinate-ammonium and bialaphos on the phytoplankton community of a small eutrophic northern lake. Environ Toxicol Chem 17: 1282–1290.

Fairchild JF, Sappington LC (2002) Fate and effects of the triazinone herbicide metribuzin in experimental pond mesocosms. Arch Environ Contam Toxicol 43: 198–202.

Faust M, Altenburger R, Boedecker W, Grimme LH (1994) Algal toxicity of binary combinations of pesticides. Bull Environ Contam Toxicol 53: 134–141.

Faust M, Altenburger R, Backhaus T, Blanck H, Boedeker W, Gramatica P, Hamer V, Scholze M, Vighi M, Grimme LH (2001) Predicting the joint algal toxicity of multi-component s-triazine mixtures at low-effect concentrations of individual toxicants. Aquat Toxicol 56: 13–32.

Filser J (2008) Ecotoxicology and ecosystems: Relevance, restrictions, research needs. Basic Appl Ecol 9: 333–336.

Francoeur SN, Johnson AC, Kuehn KA, Neely RK (2007) Evaluation of the efficacy of the photosystem II inhibitor DCMU in periphyton and its effects on nontarget microorganisms and extracellular enzymatic reactions. J N Am Benthol Soc 26: 633–641.

Geiszinger A, Bonnineau C, Faggiano L, Guasch H, Lopez-Daval J, Proia L, Ricart M, Ricciardi F, Romaní A, Rotter S, Muñoz I, Schmitt-Jansen M, Sabater S (2009). The relevance of the community approach linking chemical and biological analyses in pollution assessment. Trends Anal Chem 28: 619–626.

Gilliom RJ (2007) Pesticides in U.S. streams and groundwater. Environ Sci Technol 15: 3409–3414.

Gruessner B, Watzin MC (1996) Response of aquatic communities from a Vermont stream to environmentally realistic atrazine exposure in laboratory microcosms. Environ Toxicol Chem 15: 410–419.

Guasch H, Ivorra N, Lehmann V, Paulsson M, Real M, Sabater S (1998a) Community composition and sensitivity of periphyton to atrazine in flowing waters: The role of environmental factors. J Appl Phycol 10: 203–213.

Guasch H, Sabater S (1998b) Light history influences the sensitivity to atrazine in periphytic algae. J Phycol 34: 233–241.

Guasch H, Admiraal W, Sabater S (2003) Contrasting effects of organic and inorganic toxicants on freshwater periphyton. Aquat Toxicol 64: 165–175.

Guasch H, Lehmann V, van Beeusekom B, Sabater S, Admiraal W (2007) Influence of phosphate on the response of periphyton to atrazine exposure. Arch Environ Contam Toxicol 52: 32–37.

Gustavson K, Wängberg SÅ (1995) Tolerance induction and succession in microalgae communities exposed to copper and atrazine. Aquat Toxicol 32: 283–302.

Gustavson K, Møhlenberg F, Schlüter L (2003) Effects of exposure duration of herbicides on natural stream periphyton communities and recovery. Arch Environ Contam Toxicol 45: 48–58.

Hartgers EM, Aalderink GHR, Van den Brink PJ, Gylstra R, Wiegman JWF, Brock TCM (1998) Ecotoxicological threshold levels of a mixture of herbicides (atrazine, diuron and metolachlor) in freshwater microcosms. Aquat Ecol 32: 135–152.

Junghans M, Backhaus T, Faust M, Scholze M, Grimme LH (2003) Predictability of combined effects of eight chloroacetanilide herbicides on algal reproduction. Pest Manag Sci 59: 1101–1110.

Jüttner I, Peither A, Lay JP, Kettrup A, Ormerod SJ (1995) An outdoor mesocosm study to assess ecotoxicological effects of atrazine on a natural plankton community. Arch Environ Contam Toxicol 29: 435–441.

Kasai F, Hanazato T (1995a) Effects of the triazine herbicide, simetryn, on freshwater plankton communities in experimental ponds. Environ Pollut 89: 197–202.

Kasai F, Hanazato T (1995b) Genetic changes in phytoplankton communities exposed to the herbicide simetryn in outdoor experimental ponds. Arch Environ Contam Toxicol 28: 154–160.

Knauer K, Leimgruber A, Hommen U, Knauert S (2010) Co-tolerance of phytoplankton communities to photosynthesis II inhibitors. Aquat Toxicol 96: 256–263.

Knauert S, Escher B, Singer H, Hollender J, Knauer K (2008) Mixture toxicity of three photosystem II inhibitors (atrazine, isoproturon, and diuron) toward photosynthesis of freshwater phytoplankton studied in outdoor mesocosms. Environ Sci Technol 42: 6424–6430.

Knauert S, Dawo U, Hollender J, Hommen U, Knauer K (2009) Effects of photosystem II inhibitors and their mixture on freshwater phytoplankton succession in outdoor mesocosms. Environ Toxicol Chem 28: 836–845.

Kobraei ME, White DS (1996) Effects of 2,4-dichlorophenoxyacetic acid on Kentucky algae: simultaneous laboratory and field toxicity testings. Arch Environ Contam Toxicol 31: 571–580.

Laviale M, Prygiel J, Créach A (2010) Light modulated toxicity of isoproturon toward natural stream periphyton photosynthesis: a comparison between constant and dynamic light conditions. Aquat Toxicol 97: 334–342.

Leboulanger C, Bouvy M, Carré C, Cecchi P, Amalric L, Bouchez A, Pagano M, Sarazin G (2011) Comparison of the effects of two herbicides and an insecticide on tropical freshwater plankton in microcosms. Arch Environ Contam Toxicol DOI 10.1007/s00244-011-9653-3

Leboulanger C, Rimet F, Hème de Lacotte M, Bérard A (2001) Effects of atrazine and nicosulfuron on freshwater microalgae. Environ Pollut 26: 131–135.

Liscio C, Magi E, Di Carro M, Suter MJF, Vermeirssen ELM (2009) Combining passive samplers and biomonitors to evaluate endocrine disrupting compounds in a wastewater treatment plant by LC/MS/MS and bioassay analyses. Environ Pollut 157: 2716–2721.

López-Doval JC, Ricart M, Guasch H, Romaní AM, Sabater S, Muñoz I (2010) Does grazing pressure modify diuron toxicity in a biofilm community? Arch Environ Contam Toxicol 58: 955–962.

McClellan K, Altenburger R, Schmitt-Jansen M (2008) Pollution-induced community tolerance as a measure of species interaction in toxicity assessment. J Appl Ecol 45: 1514–1522.

Mohr S, Schröder H, Feibicke M, Berghahn R, Arp W, Nicklisch A (2008a) Long-term effects of the antifouling booster biocide irgarol 1051 on periphyton, plankton and ecosystem function in freshwater pond mesocosms. Aquat Toxicol 90: 109–120.

Mohr S, Feibicke M, Berghahn R, Schmiediche R, Schmidt R (2008b). Response of plankton communities in freshwater pond and stream mesocosms to the herbicide metazachlor. Environ Pollut 152: 530–542.

Montuelle B, Dorigo U, Bérard A, Volat B, Bouchez A, Tlili A, Gouy V, Pesce S (2010) The periphyton as a multimetric bioindicator for assessing the impact of land use on rivers: an overview of the Ardières-Morcille experimental watershed (France). Hydrobiologia 657: 123–141.

Morin S, Bottin M, Mazzella N, Macary F, Delmas F, Winterton P, Coste M (2009) Linking diatom community structure to pesticide input as evaluated through a spatial contamination potential (Phytopixal): a case study in the Neste river system (South-West France). Aquat Toxicol 94: 28–39.

Morin S, Pesce S, Tlili A, Coste M, Montuelle B (2010) Recovery potential of periphytic communities in a river impacted by a vineyard watershed. Ecol Indic 10: 419–426.

Muller R, Tang JYM, Thier R, Mueller JF (2007). Combining passive sampling and toxicity testing for evaluation of mixtures of polar organic chemicals in sewage treatment plant effluent. J Environ Monit 9: 104–109.

Muñoz I, Real M, Guasch H, Navarro E, Sabater S (2001) Effects of atrazine on periphyton under grazing pressure. Aquat Toxicol 55: 239–249.

Noack U, Geffke T, Balasubramanian R, Papenbrock J, Braune M, Scheerbaum D (2003) Effects of the herbicide metazachlor on phytoplankton and periphyton communities in outdoor mesocosms. Acta Hydrochim Hydrobiol 31: 482–490.

Nyström B, Paulsson M, Almgren K, Blanck H (2000) Evaluation of the capacity for development of atrazine tolerance in periphyton from a Swedish freshwater site as determined by inhibition of photosynthesis and sulfolipid synthesis. Environ Toxicol Chem 19: 1324–1331.

Nyström B, Becker-Van Slooten K, Bérard A, Grandjean D, Druart JC, Leboulanger C (2002) Toxic effects of Irgarol 1051 on phytoplankton and macrophytes in Lake Geneva. Water Res 36: 2020–2028.

Pannard A, Le Rouzic B, Binet F (2009) Response of phytoplankton community to low-dose atrazine exposure combined with phosphorus fluctuations. Arch Environ Contam Toxicol 57: 50–59.

Pearson N, Crossland NO (1996) Measurement of community photosynthesis and respiration in outdoor artificial streams. Chemosphere 32: 913–919.

Pérès F, Florin D, Grollier T, Feurtet-Mazel A, Coste M, Ribeyre F, Ricard M, Boudou A (1996) Effects of the phenylurea herbicide isoproturon on periphytic diatom communities in freshwater indoor microcosms. Environ Pollut 94: 141–152.

Pérez GL, Torremorell A, Mugni H, Rodriguez P, Solange Vera M, do Nascimento M, Allende L, Bustingorry J, Escaray R, Ferraro M, Izaguirre I, Pizarro H, Bonetto C, Morris DP, Zagarese H (2007) Effects of the herbicide Roundup on freshwater microbial communities: a mesocosm study. Ecol Appl 17: 2310–2322.

Perschbacher PW, Stone N, Ludwig GM, Guy Jr CB (1997) Evaluation of effects of common aerially-applied soybean herbicides and propanil on the plankton communities of aquaculture ponds. Aquaculture 157: 117–122.

Perschbacher PW, Ludwig GM, Slaton N (2002) Effects of common aerially-applied rice herbicides on the plankton communities of aquaculture ponds. Aquaculture 214: 241–246.

Perschbacher PW, Ludwig GM (2004) Effects of diuron and other aerially-applied cotton herbicides and defoliants on the plankton communities of aquaculture ponds. Aquaculture 233: 197–203.

Perschbacher PW, Ludwig GM, Edziyie R (2008) Effects of atrazine drift on production pond plankton communities and water quality using experimental mesocosms. J World Aquacult Soc 39: 126–130.

Pesce S, Fajon C, Bardot C, Bonnemoy F, Portelli C, Bohatier J (2006) Effects of the phenylurea herbicide diuron on natural riverine microbial communities in an experimental study. Aquat Toxicol 78: 303–314.

Pesce S, Fajon C, Bardot C, Bonnemoy F, Portelli C, Bohatier J (2008) Longitudinal changes in microbial planktonic communities of a French river in relation to pesticide and nutrient inputs. Aquat Toxicol 86: 352–360.

Pesce S, Batisson A, Bardot C, Fajon C, Portelli C, Montuelle B, Bohatier J (2009) Response of spring and summer riverine microbial communities following glyphosate exposure. Ecotoxicol Environ Saf 72: 1905–1912.

Pesce S, Margoum C, Montuelle B (2010a) In situ relationships between spatio-temporal variations in diuron concentrations and phototrophic biofilm tolerance in a contaminated river. Water Res 44: 1941–1949.

Pesce S, Lissalde S, Lavieille D, Margoum C, Mazzella N, Roubeix V, Montuelle B (2010b) Evaluation of single and joint toxic effects of diuron and its main metabolites on natural phototrophic biofilms using a pollution-induced community tolerance (PICT) approach. Aquat Toxicol 99: 492–499.

Pesce S, Morin S, Lissalde S, Montuelle B, Mazzella N (2011) Combining polar organic chemical integrative samplers (POCIS) with toxicity testing to evaluate pesticide mixture effects on natural phototrophic biofilms. Environ Pollut 159: 735–741.

Pinckney JL, Örnólfsdóttir EB, Lumsden E (2002) Estuarine phytoplankton group-specific responses to sublethal concentrations of the agricultural herbicide, atrazine. Mar Pollut Bull 44: 1109–1116.

Pollehne F, Jost G, Kerstan E, Meyer-Harms B, Reckerman M, Nausch M, Wodarg D (1999) Triazine herbicides and primary pelagic interactions in an estuarine summer situation. J Exp Mar Biol Ecol 238: 243–257.

Rabiet M, Margoum C, Gouy V, Carluer N, Coquery M (2009) Assessing pesticide concentration dynamics and fluxes in the stream of a small vineyard watershed - Effect of sampling strategy. Environ Pollut 158: 737–748.

Relyea RA (2005) The impact of insecticides and herbicides on the biodiversity and productivity of aquatic communities. Ecol Appl 15: 618–627.
Relyea R, Hoverman J (2006) Assessing the ecology in ecotoxicology: a review and synthesis in freshwater systems. Ecol Lett 9: 1157–1171.
Relyea RA (2009) A cocktail of contaminants: how mixtures of pesticides at low concentrations affect aquatic communities. Oecologia 159: 363–376.
Ricart M (2011) Effects of priority and emerging pollutants on river biofilms, phD Thesis, University of Girona, Spain, 202 p.
Ricart M, Barceló D, Geiszinger A, Guasch H, Alda MLD, Romaní AM, Vidal G, Villagrasa M, Sabater S (2009) Effects of low concentrations of the phenylurea herbicide diuron on biofilm algae and bacteria. Chemosphere 76: 1392–1401.
Ricart M, Guasch H, Barceló D, Brix R, Conceição MH, Geiszinger A, López de Alda MJ, López-Doval JC, Muñoz I, Postigo C, Romaní AM, Villagrasa M, Sabater S (2010) Primary and complex stressors in polluted Mediterranean rivers: Pesticide effects on biological communities. J Hydrol 383: 52–61.
Rohr JR, Crumrine PW (2005) Effects of an herbicide and an insecticide on pond community structure and processes. Ecol Appl 15: 1135–1147.
Rotter S, Sans-Piché F, Streck G, Altenburger R Schmitt-Jansen M (2011) Active bio-monitoring of contamination in aquatic systems – An in situ translocation experiment applying the PICT concept. Aquat Toxicol 101, 228–236.
Sánchez P, Kubitza J, Dohmen GP, Tarazona JV (2006) Aquatic risk assessment of the new rice herbicide profoxydim. Environ Pollut 142, 181–189.
Schaffer JD, Sebetich MJ (2004) Effects of aquatic herbicides on primary productivity of phytoplankton in the laboratory. Bull Environ Contam Toxicol 72: 1032–1037.
Schmitt-Jansen M, Altenburger R (2005a) Predicting and observing responses of algal communities to photosystem II-herbicide exposure using pollution-induced community tolerance and species-sensitivity distributions. Environ Toxicol Chem 24: 304–312.
Schmitt-Jansen M, Altenburger R (2005b) Toxic effects of isoproturon on periphyton communities – a microcosm study. Estuar Coast Shelf Sci 62: 539–545.
Schmitt-Jansen M, Altenburger R (2008) Community-level microalgal toxicity assessment by multiwavelength-excitation PAM fluorometry. Aquat Toxicol 86: 49–58.
Schmitt-Jansen M, Veit U, Dudel G, Altenburger R (2008) An ecological perspective in aquatic ecotoxicology: Approaches and challenges. Basic Appl Ecol 9: 337–345.
Schneider J, Morin A, Pick FR (1995) The response of biota in experimental stream channels to a 24-hour exposure to the herbicide Velpar L®. Environ Toxicol Chem 14, 1607–1613.
Schuler LJ, Rand GM (2008) Aquatic risk assessment of herbicides in freshwater ecosystems of South Florida. Arch Environ Contam Toxicol 54: 571–583.
Seguin F, Leboulanger C, Rimet F, Druart JC, Bérard A (2001) Effects of atrazine and nicosulfuron on phytoplankton in systems of increasing complexity. Arch Environ Contam Toxicol 40: 198–208.
Seguin F, Le Bihan F, Leboulanger C, Bérard A (2002) A risk assessment of pollution: induction of atrazine tolerance in phytoplankton communities in freshwater outdoor mesocosms, using chlorophyll fluorescence as an endpoint. Wat Res 36: 3227–3236.
Shaw M, Negri A, Fabricius K, Mueller JF (2009). Predicting water toxicity: Pairing passive sampling with bioassays on the Great Barrier Reef. Aquat Toxicol 95: 108–116.
Sinclair CJ, Boxall ABA (2003) Assessing the ecotoxicity of pesticide transformation products. Environ Sci Technol 37: 4617–4625.
Slijkerman DME, Moreira-Santos M, Robbert GJ, Ribeiro R, Soares AMVM, van Straalen NM (2005) Functional and structural impact of linuron on a freshwater community of primary producers: The use of immobilized algae. Environ Toxicol Chem 24: 2477–2485.
Solomon KR, Baker DB, Richards RP, Dixon KR, Klaine SJ, La Point TW, Kendall RJ, Weisskopf CP, Giddings JM, Giesy JP, Hall LW, Williams WM (1996) Ecological risk assessment of atrazine in North American surface waters. Environ Toxicol Chem 15: 31–76.

Spawn RL, Hoagland KD, Siegfried BD (1997) Effects of alachlor on an algal community from a midwestern agricultural stream. Environ Toxicol Chem 16: 785–793.

Takahashi Y, Houjyo T, Kohjimoto T, Takagi Y, Mori K, Muraoka T, Annoh H, Ogiyama K, Funaki Y, Tanaka K, Wada Y, Fujita T (2007) Impact of pretilachlor herbicide and pyridaphenthion insecticide on aquatic organisms in model streams. Ecotoxicol Environ Safe 67: 227–239.

Tlili A, Dorigo U, Montuelle B, Margoum C, Carluer N, Gouy V, Bouchez A, Bérard A (2008) Responses of chronically contaminated biofilms to short pulses of diuron: An experimental study simulating flooding events in a small river. Aquat Toxicol 87: 252–263.

Tlili A, Bérard A, Roulier JL, Volat B, Montuelle B (2010) PO_4^{3-} dependence of the tolerance of autotrophic and heterotrophic biofilm communities to copper and diuron. Aquat Toxicol 98: 165–177.

Tlili A, Montuelle B (2011) Microbial Pollution-Induced Community Tolerance, in: Amiard-Triquet C, Rainbow PS, Roméo M (Eds.) Tolerance to Environmental Contaminants. CRCpress. p. 85–108.

Tlili A, Montuelle B, Bérard A, Bouchez A (2011) Impact of chronic and acute pesticide exposures on periphyton communities. Sci Total Environ 409: 2102–2113

Traunspurger W, Schäfer H, Remde A (1996) Comparative investigation on the effect of a herbicide on aquatic organisms in single species tests and aquatic microcosms. Chemosphere 33: 1129–1141.

Valiente Moro C, Crouzet O, Rasconi S, Thouvenot A, Coffe G, Batisson I, Bohatier J (2009) New design strategy for development of specific primer sets for PCR-based detection of *Chlorophyceae* and *Bacillariophyceae* in environmental samples. Appl Environ Microbiol 75: 5729–5733.

Van den Brink PJ, Van Donk E, Gylstra R, Crum SJH, Brock TCM (1995) Effect of chronic low concentrations of the pesticides chlorpyrifos and atrazine in indoor freshwater microcosms. Chemosphere 31: 3181–3200.

Van den Brink PJ, Hartgers EM, Fettweis U, Crum SJH, Van Donk E, Brock TCM (1997) Sensitivity of macrophyte-dominated freshwater microcosms to chronic levels of the herbicide linuron. Ecotox Environ Safe 38: 13–24.

Van den Brink PJ, Crum SJH, Gylstra R, Bransen F, Cuppen JGM, Brock TCM (2009) Effects of a herbicide-insecticide mixture in freshwater microcosms: Risk assessment and ecological effect chain. Environ Pollut 157: 237–249.

Van Geest GJ, Zwaardemaker NG, Van Wijngaarden RPA, Cuppen JGM (1999) Effects of a pulsed treatment with the herbicide afalon (active ingredient linuron) on macrophyte-dominated mesocosms. II. Structural responses. Environ Toxicol Chem 18: 2866–2874.

Van Wijngaarden RPA, Cuppen JGM, Arts GHP, Crum SJH, van den Hoorn MW, van den Brink PJ, Brock TCM (2004) Aquatic risk assessment of a realistic exposure to pesticides used in bulb crops: A microcosm study. Environ Toxicol Chem 23: 1479–1498.

Vera MS, Lagomarsino L, Sylvester M, Pérez GL, Rodríguez P, Mugni H, Sinistro R, Ferraro M, Bonetto C, Zagarese H, Pizarro H (2010) New evidences of Roundup® (glyphosate formulation) impact on the periphyton community and the water quality of freshwater ecosystems. Ecotoxicology 19: 710–721.

Villeneuve A, Montuelle B, Bouchez A (2011) Effects of flow regime and pesticides on periphytic communities: evolution and role of biodiversity. Aquat Toxicol 102:123–133.

Waiser MJ, Robarts RD (1997) Impacts of a herbicide and fertilizers on the microbial community of a saline prairie lake. Can J Fish Aquat Sci 54: 320–329.

Wellmann P, Ratte HT, Heimbach F (1998) Primary and secondary effects of methabenzthiazuron on plankton communities in aquatic outdoor microcosms. Aquat Ecol 32: 125–134.

Wendt-Rasch L, Pirzadeh P, Woin P (2003) Effects of metsulfuron methyl and cypermethrin exposure on freshwater model ecosystems. Aquat Toxicol 63: 243–256.

Wendt-Rasch L, Van den Brink PJ, Crum SJH, Woin P (2004) The effects of a pesticide mixture on aquatic ecosystems differing in trophic status: responses of the macrophyte *Myriophyllum spicatum* and the periphytic algal community. Ecotox Environ Safe 57: 383–398.

Woudneh MB, Ou Z, Sekela M, Tuominen T, Gledhill M (2009) Pesticide multiresidues in waters of the lower Fraser valley, British Columbia, Canada. Part I. Surface water. J Environ Qual 38: 940–947.

Essential Roles and Hazardous Effects of Nickel in Plants

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1 Introduction

Nickel is one of 23 metal pollutants of great concern to the environment and to human health (Sunderman 1992; Jarup 2003; Duda-Chodak and Baszczyk 2008). Nickel is the 24th most abundant element (twice as Cu) and comprises approximately 0.008% of the content of the earth's crust; hence, it is a natural component of soil (parent material) and water (Alloway 1995; Hostýnek and Maibach 2002; Hedfi et al. 2007). Most of the earth's nickel, however, is inaccessible, as it is locked in the iron–nickel molten core, which constitutes approximately 10% nickel. The second largest Ni deposits of the earth rest in the sea. It is estimated that the sea contains approximately eight billion tons of Ni, either dissolved in seawater or deposited in the seabed (Birch 1964; Stixrude et al. 1997). Soils may contain nickel levels as low as 0.2 mg kg^{-1} or as high as 450 mg kg^{-1}. The average nickel content in soil is approximately 20 mg kg^{-1}; however, the content level may vary greatly depending upon the mode of origin of the soil's parent material (Assembly of Life Sciences 1975; Aubert and Pinta 1978; Wilson and Kordybach 2000). Because organic matter strongly absorbs some metals, particularly nickel, fossil fuels such as coal and oil may contain considerable amounts of nickel (Sigel et al. 2005). Moreover, Ni naturally occurs in a few plants (legumes) where it functions as an essential component of some enzymes (e.g., ureases) that are involved in nitrogen assimilation (Eskew et al. 1984; Brown et al. 1987a; Sakamoto and Bryant 2001). Tea plants may contain high Ni levels (at concentrations up to 5.3 mg kg^{-1} in dried leaves), whereas Ni levels in other plants vary: instant tea (15.5 mg kg^{-1}), cacao powder (9.8 mg kg^{-1}), cashews (5.1 mg kg^{-1}), soy protein (4.3 mg kg^{-1}), walnuts (3.6 mg kg^{-1}), filberts and peanuts (1.6 mg kg^{-1}), almonds (1.3 mg kg^{-1}), wheat germ (1 mg kg^{-1}), pistachios (0.8 mg kg^{-1}), and rice (0.4 mg kg^{-1}) (Nielsen 1993). The nickel content in some vegetables varies from 0.26 mg kg^{-1} (beans) to 0.08 mg kg^{-1} (tomatoes). Some fruits, such as peaches (0.16 mg kg^{-1}) and apples (0.03 mg kg^{-1}), may also contain moderate amounts of nickel (Nielsen 1993).

The National Pollutant Inventory (NPI) of the Government of Australia ranked 400 hazardous materials in order of their relative potential hazards and probable exposure levels. Nickel and its compounds were ranked as number 54 (National Pollutant Inventory 1999) on this list. In another ranking by the same organization, environmental contaminants were ranked on a scale of 0–3, based on (1) the extent of their toxic or poisonous nature, (2) their ability to remain active as an environmental pollutant, and (3) their ability to accumulate in living organisms. Nickel and its compounds were rated at 1.2 as a health hazard and 1.0 as an environmental hazard (National Pollutant Inventory 1999).

Nickel has long been known as an important plant micronutrient, and for having a multitude of biological functions (Eskew et al. 1983; Kochian 1991; Welch 1995;

Hasinur et al. 2005). Therefore, any Ni deficiency negatively affects plant growth and metabolism in many ways. Such deficiency may affect (a) plant growth, (b) plant senescence, (c) N metabolism, and (d) Fe uptake. Ni-deficient plants may develop chlorosis in the youngest leaves, and ultimately Ni deficiency may produce meristematic necrosis (Brown et al. 1987b, 1990; Yossef et al. 1998; Bai et al. 2006a; Wood et al. 2006). In addition, Ni is a constituent of several metalloenzymes, e.g., urease (Eskew et al. 1984; Brown et al. 1987a; Sakamoto and Bryant 2001). Therefore, a deficiency of Ni leads to reduced urease activity and disturbs N assimilation (Ermler et al. 1998; Küpper and Kroneck 2007). In addition to the foregoing functions, Ni is also proposed to participate in several important metabolic reactions (e.g., hydrogen metabolism, methane biogenesis, and acetogenesis in bacteria) (Maier et al. 1993; Collard et al. 1994; Ragsdale 1998; Mulrooney and Hausinger 2003). Finally, nickel is known to have a role in phytoalexin synthesis and in plant resistance to various plant stresses (Graham et al. 1985; Barker 2006; Wood and Reilly 2007).

Although small Ni concentrations are essential to normal plant growth, high concentrations may exert deleterious effects on plant growth and produce symptoms of toxicity. The symptoms associated with nickel toxicity to plants include the following: reduced shoot and root growth, poor development of the branching system, deformation of various plant parts and abnormal flower shape, decreased biomass production, leaf spotting, mitotic root tip disturbances, inhibition of germination, and chlorosis that can result in foliar necrosis (Ewais 1997; Rao and Sresty 2000; Pandey and Sharma 2002; Nakazawa et al. 2004; Rahman et al. 2005; Gajewska et al. 2006). All of these toxic effects ultimately reduce the yield of agricultural crops (Balaguer et al. 1998; Ahmad et al. 2007). Other symptoms of Ni plant toxicity include Fe deficiency-induced chlorosis and foliar necrosis (Brown et al. 1987a; Wood et al. 2006). Excess nickel affects nutrient absorption by roots, plant development and metabolism, and inhibits photosynthesis and transpiration (Nedhi et al. 1990; Kochian 1991; Pandey and Sharma 2002; Hasinur et al. 2005; Rahman et al. 2005). Nickel also has the ability to replace Co and certain other heavy metals located at active sites in metalloenzymes and can, thereby, disrupt their functioning (Ermler et al. 1998; Küpper and Kroneck 2007).

In view of the foregoing, it is amply clear that Ni enters the environment from several sources, and although it is beneficial for optimum plant growth at low concentrations, it may produce hazardous effects on growth and key metabolic processes of most plants at high concentrations. It is our purpose in the present review to address both the beneficial and toxic effects that Ni displays in plants. Moreover, we also address the key processes used by plants, whether morphoanatomical or metabolic, that enables them to tolerate excessive Ni levels.

2 Sources of Nickel Emission to the Environment

Nickel is released into the environment from a variety of natural and anthropogenic sources. Among industrial sources, a considerable amount of environmental Ni derives from the combustion of coal, oil, and other fossil fuels. Other industrial

sources that contribute to nickel emissions are mining and refining processes, nickel alloy manufacturing (steel), electroplating, and incineration of municipal wastes (Sharma 2005; Ensink et al. 2007). Wastewater from municipal sewage treatment plants also contributes to environmental metal accumulation (van der Hoek et al. 2002).

Ni has many uses in modern society. Nickel is most commonly used in the production of Ni-based alloys (e.g., steel). Currently, more than 3,000 nickel-based alloys are used in industry, agriculture, and households (Sigel and Sigel 1994). Several nickel compounds are put to essential industrial or other uses. Nickel acetate is a substance mainly used in the textile industry as a mordant, as a hydrogenation catalyst during vegetable ghee production, and in nickel-based electroplating. Nickel hydroxide is used in nickel–cadmium (rechargeable) batteries. Nickel carbonate is used in vacuum tubes and transistor cans, as a catalyst for removing organic contaminants present in wastewater, for preparation of colored glass, and in nickel electroplating. Nickel oxide is used in fuel cell electrodes, for electroplating, for the production of active nickel catalysts, and in coloring and decolorizing glass products (Dennis and Such 1993; Cempel and Nikel 2006). Some loss of Ni to the environment undoubtedly occurs from all of these uses.

Another important source of Ni emission is from power generating plants and trash incinerators. These contribute to the release of nickel primarily to the air (Hilgenkamp 2005; Lewinsky 2007). The removal of nickel from the atmosphere is not easy to achieve and generally takes a long time (Nriagu 1989). Ni residues suspended in air usually settle to the ground by dry deposition, or may fall as a component of precipitation, following a series of wet reactions (Galloway et al. 1982). Ultimately, nickel is deposited on natural or other surfaces and reaches groundwater (Long et al. 1995). Usually, the larger portion of nickel and its compounds that are released to the environment are adsorbed onto sediments or soil particles and thus becomes immobile (Nriagu and Pacyna 1988; Smith et al. 1996). However, in acidic soils its solubility increases and it becomes more mobile (Zhang et al. 2006). When this occurs, Ni often leaches to the groundwater (Sullivan and Krieger 2001). Because Ni reaches the environment from a variety of sources, most living organisms become exposed to its toxic effects at one point or another during their lives.

3 Nickel Availability and Uptake

Nickel uptake in plant root systems occurs via passive diffusion, as well as by active transport (Seregin and Kozhevnikova 2006; Chen et al. 2009). However, the ratio of uptake through passive vs. active transport mechanisms varies greatly with (1) plant species, (2) the form of Ni present, and (3) the soil or nutrient solution concentration (Vogel-Mikus et al. 2005). For example, soluble Ni compounds are preferentially absorbed passively via a cation transport system. By contrast, chelated Ni compounds are taken up through a secondary active transport method, as some transport proteins (e.g., permeases) specifically bind Ni (Wolfram et al. 1995; Eitinger and

Mandrand-Berthelot 2000). However, insoluble Ni compounds enter plant cells mainly via endocytosis (Costa et al. 1994).

The factors that influence Ni bioavailability and uptake by plants include the following: (1) soil concentrations of nickel (Cataldo et al. 1978), (2) acidity of the soil or the soil solution (McIlveen and Negusanti 1994; Antoniadis et al. 2008), (3) presence of other competitive metals (Kochian 1991), and (4) organic composition of the soil (Burke et al. 2000; Jean et al. 2008). Among these, the most important factor that determines Ni solubility is soil pH, and this, in turn, determines nickel's availability for plant uptake (Smith 1994; Weng et al. 2004; Antoniadis et al. 2008). Therefore, anthropogenic processes that change soil pH alter Ni solubility in soils. For example, at low pH in acidic soils Ni is in a more soluble form and thus becomes more mobile (Zhang et al. 2006). Therefore, symptoms of metal toxicity to plants can easily be observed in acid soils, sometimes even if no other metal is naturally present or is added to the soil system by human activities (Rautaray et al. 2003).

Soil colloidal materials have the ability to absorb nickel. Nickel is present in an exchangeable form when bound to organic matter and can, therefore, be easily exchanged with the crystal lattice of minerals in the soil solid phase (Misra and Pande 1974; Karaca 2004; Sukkariyah et al. 2005). Although Ni solubility in soils increases with soil acidity, high Ni mobility may also occur under neutral or alkaline conditions (Willaert and Verloo 1988; Alloway 1995). Generally, mobile forms of nickel are readily available for uptake and most of the nickel ion absorbed by plants accumulates primarily in roots. Still, Ni, in some cases, is known to translocate to aerial plant parts in some species. Hence, uptake and accumulation of Ni is species-, pH-, amount-, and available-form-dependent.

4 Transport and Distribution in Plants

Nickel and certain other metals are primarily transported from roots to shoots (Peralta-Videa et al. 2002) and to leaves (Krupa et al. 1993) via the xylem, through the transpiration stream (Neumann and Chamel 1986). Nickel is highly mobile in plants and can be easily retranslocated from old to young leaves (Zhao et al. 1999; Gray and Mclaren 2006). As it is an essential element, Ni may also be translocated via the phloem to neonatal plant parts, such as buds, fruits, and seeds (McIlveen and Negusanti 1994; Welch 1995; Fismes et al. 2005; Page et al. 2006). Such transport and retranslocation is strongly regulated by metal–ligand complexes (i.e., nicotianamine, histidine, and organic acids) (Vacchina et al. 2003; Kim et al. 2005; Pianelli et al. 2005; Haydon and Cobbett 2007) and by proteins that specifically bind and transport Ni (Hausinger 1997; Colpas and Hausinger 2000).

Approximately, half of the Ni taken up by plants is retained in the plant root system (Cataldo et al. 1978). This may result from its sequestration at cation exchange sites of vessel walls, xylem parenchyma cells and/or immobilization in the root (Seregin and Kozhevnikova 2006). Furthermore, root vascular cylinders contain a high percentage of Ni (over 80%), while less than 20% is present in the

corticular region. This distribution may explain why Ni has good mobility in xylem and phloem tissues (Marschner 1995; Page and Feller 2005; Riesen and Feller 2005). However, the distribution of Ni varies in stems and leaves, where it is distributed preferentially in epidermal cells, and probably in vacuoles, rather than in the cell wall (Küpper et al. 2001). The distribution of nickel in leaf organelles and in the cytoplasm is different. Herein, Ni's distribution is higher in the cytoplastic fluid and in vacuoles (where it may exceed 87%), and lower in chloroplasts (a content of 8–9.9%), mitochondria, and ribosomes (which contain 0.32–2.85% of total nickel) (Brooks et al. 1981).

Nickel is translocated to fruits and seeds through the phloem (McIlveen and Negusanti 1994; Welch 1995; Fismes et al. 2005; Page et al. 2006). However, its distribution within the seed varies greatly, depending upon the plant species involved, and on other factors such as presence of pathogens or insects (Boyd et al. 2006). For example, in the seeds of *Stackhousia tryonii*, a metal hyperaccumulator species, Ni partitioned to the pericarp (fruit wall), while little entered endospermic and cotyledonary tissues. However, the high amounts of Ni that partitioned into the fruit wall had no effect on seed germination of *S. tryonii* (Bhatia et al. 2003). Thus, exclusion of Ni from embryonic tissues may ensure high reproductive success of such hyperaccumulating species when they are grown on metal-enriched soils.

5 Role of Nickel as a Micronutrient

The discovery of nickel's metabolic role in plants occurred early in the twentieth century. At that time nickel was discovered to be a constituent of plant tissue ash residue. The evidence for Ni's metabolic role was not discovered until Roach and Barclay (1946) disclosed the results of their research into this topic. Later, Dobrolyubskii and Slavvo (1957) further strengthened the understanding of nickel's metabolic role in plants, as has many more recently published papers (Shimada et al. 1980; Welch 1981; Eskew et al. 1983, 1984; Brown et al. 1987a, b, 1990; Gerendás et al. 1999; Gerendás and Sattelmacher 1997a; Mulrooney and Hausinger 2003; Wood et al. 2006).

Nickel's role as an essential plant nutrient came from the work of Dixon et al. (1975), who proved the essentiality of it to the urease activity of Jack-bean (urea amidohydrolase, EC 3.5.1.5). This entity is a metalloenzyme that requires nickel as an integral part for its proper functioning. Since this discovery, nickel has proven to be an essential micronutrient for proper functioning of many other metalloenzymes (Klucas et al. 1983; Brown et al. 1987a; Sakamoto and Bryant 2001). It is estimated that there are ~500 proteins and peptides in living system that have the ability to bind Ni (Maier et al. 2007). Therefore, Ni is now understood to be an integral component of many biomolecules (including metalloenzymes), where its presence is required to maintain normal structure and functioning (Won and Lee 2004; Seregin and Kozhevnikova 2006; Benoit et al. 2007). The following are major enzymes that require Ni for catalysis, either in lower or higher plants: *urease, superoxide dismutase, NiFe hydrogenases,*

Table 1 The name and function of selected key enzymes in bacterial, fungal, and plant species that are affected by nickel

Species	References
1. ***Urea amidohydrolase (Urease) (EC. 3.5.1.5)***: catalyzes the hydrolysis of urea to release two molecules of ammonia and one molecule of carbon dioxide (Klucas et al. 1983).	
Glycine max (Soybean)	Polacco (1976, 1977), Polacco and Havir (1979), Polacco et al. (1982), Polacco and Sparks (1982), Klucas et al. (1983), Eskew et al. (1984), Polacco and Winkler (1984) Dalton et al. (1985)
Canavalia ensiformis (Jack bean)	Dixon et al. (1975), Blakely and Zerner (1984)
Pisum sativum (Pea)	Horak (1985)
Oryza sativa (Rice)	Gerendás et al. (1998), Moraes et al. (2009)
Cucurbita pepo (Pumpkin)	Gerendás and Sattelmacher (1997a)
Elymus condensatus (Rye)	Gerendás and Sattelmacher (1997b)
Carya illinoinensis (Pecan)	Bai et al. (2006a, b, 2007), Reilly et al. (2006)
Vigna unguiculata (Cowpeas)	Eskew et al. (1984)
Lemna paucicostata (Water lentil)	Gordon et al. (1978)
Lycopersicon esculentum (Tomato)	Shimada et al. (1980), Tan et al. (2000)
Brassica napus (Rapeseed)	Gerendás and Sattelmacher (1999)
Aspergillus nidulans	Mackay and Pateman (1980)
Thiocapsa roseopersicina	Bast (1988)
Chromatium vinosum	Bast (1988)
Thiocystis violacea	Bast (1988)
Phaeodactylum tricornutum	Rees and Bekheet (1982)
Tetraselmis subcordiformis	Rees and Bekheet (1982)
Helicobacter pylori	Olson et al. (2001), Benoit and Maier (2003), Mehta et al. (2003)
Evernia prunastri	Pérezurria et al. (1986)
2. ***Ni-Superoxide dismutase (EC 1.15.1.1)***: involves the binding of superoxide to the Ni^{2+} center, generating a Ni^{2+}-peroxo species that undergoes proton and electron transfer to generate H_2O_2 and the oxidized Ni^{3+} center. Binding of a second superoxide generates a Ni^{3+}-peroxo intermediate, which donates an electron back to the Ni^{3+} center to liberate O_2 and reform the starting Ni^{2+} state (Ragsdale 2009).	
Triticum aestivum (Wheat)	Gerendás and Sattelmacher (1997b)
Glycine max Soybean	Gerendás and Sattelmacher (1997b)
Brassica napus (Rapeseed)	Gerendás and Sattelmacher (1997b)
Cucurbita pepo (Pumpkin)	Gerendás and Sattelmacher (1997b)
Helianthus annuus (Sunflower)	Gerendás and Sattelmacher (1997b)
Streptomyces seoulensis	Youn et al. (1996a), Lee et al. (2002b), Schmidt et al. (2007)
Streptomyces griseus	Youn et al. (1996b)
Prochlorococcus marinus	Eitinger (2004)
Streptomyces coelicolor	Kim et al. (1996)
Streptomyces acidiscabies	Schmidt et al. (2007)
Streptomyces peucetius	Kanth et al. (2010)
NiFe hydrogenases (EC 1.18.99.1): catalyze the reversible oxidation of molecular hydrogen (H_2); play a vital role in anaerobic microbial metabolism (Lubitz et al. 2007).	
Rhizobium japonicum	Klucas et al. (1983), Dalton et al. (1985), Soom et al. (1993)

(continued)

Table 1 (continued)

Species	References
Helicobacter pylori	Olson et al. (2001), Mehta et al. (2003)
Clostridium thermoaceticum	Drake et al. (1980), Ragsdale (2002)
Methanobacterium thermoautotrophicum	Diekert et al. (1980), Tersteegen and Hedderich (1999)
Alcaligenes eutrophus	Friedrich et al. (1982), Burgdorf et al. (2005)
Desulfovibrio gigas	Rodrigues et al. (2003)
Hydrogenobacter thermop	Ishii et al. (2000)
Desulfovibrio vulgaris	Ogata et al. 2002
Methyl CoM reductase (MCR;Coenzyme-B sulfoethylthiotransferase) (EC 2.8.4.1): all biologically generated methane on earth derives from the catalytic activity of MCR in methanogenic microbes. It also appears to catalyze anaerobic methane oxidation. MCR catalyzes the conversion of methylcoenzyme M (methyl-SCoM) and N-7- mercaptoheptanoylthreonine phosphate (CoBSH) to methane and the CoB-SS-CoM heterodisulfide (Ragsdale 2003; Jaun and Thauer 2007).	
Methanobacterium thermoautotrophicum	Ermler et al. (1997), Mahlert et al. (2002), Krüge et al. (2003)
Methanopyrus kandleri	Rospert et al. (1991)
Carbon monoxide (CO) dehydrogenase (EC 1.2.99.2): catalyzes the reversible oxidation of CO to CO_2, allowing anaerobic microbes to grow with CO or CO_2 as their sole carbon source and with CO as the only energy supply (Lindahl and Graham 2007)	
Methanobrevibacter arboriphilicus	Hammel et al. (1984)
Clostridium thermoaceticum	Diekert and Thauer (1980), Diekert and Ritter (1983), Ragsdale et al. (1983a, 1985)
Rhodospirillum rubrum	Bonam and Ludden (1987)
Methanosarcina thermophila	Terlesky et al. (1986)
Acetobacterium woodii	Ragsdale et al. (1983b)
Methanosarcina barkeri	Krzycki and Zeikus (1984), Grahame and Stadtman (1987), Krzycki et al. (1989)
Methanococcus vannielii	DeMoll et al. (1987)
Methanothrix soehngenii	Jetten et al. (1989, 1991), Eggen et al. (1991)
Acetyl CoA synthase (EC 6.2.1.1): interacts tightly with CODH to form a heterotetrameric ($\alpha 2\beta 2$) machine that couples to catalyze acetyl-CoA synthesis from CO_2, a methyl group donated by the methylated corrinoid iron-sulfur protein (CFeSP), and CoA (Doukov et al. 2008).	
Moorella thermoacetica	Darnault et al. (2003), Seravalli et al. (2004)
Carboxydothermus hydrogenoformans	Svetlitchnyi et al. (2004)
Methanosarcina thermophila	Abbanat and Ferry (1991)
Clostridium thermoaceticum	Morton et al. (1991)
Glyoxylase I (EC: 4.4.1.5): catalyzes conversion of methylglyoxal, a toxic species that forms covalent adducts with DNA, to lactate (Sukdeo et al. 2007).	
Escherichia coli	Clugston et al. (1998), He et al. (2000)
Yersinia pestis	Clugston and Honek (2000)
Pseudomonas putida	Clugston and Honek (2000)
Ni-Acireductone dioxygenase (EC 1.13.11.53): performs the penultimate step in the methionine salvage pathway (Pochapsky et al. 2007)	
Oryza sativa (Rice)	Lin et al. (2005), Sauter et al. (2005)
Klebsiella pneumoniae	Al-Mjeni et al. (2002), Pochapsky et al. (2002), Chai et al. (2008)

methyl coenzyme M reductase, carbon monoxide dehydrogenase, acetyl coenzyme-A synthase, hydrogenases, and *RNase-A* (Ermler et al. 1998; Brown 2006; Küpper and Kroneck 2007; Ragsdale 2009). A list of various Ni-dependent enzymes is presented in Table 1. Any deficiency of nickel in plants is likely to disrupt specific metabolic phenomena, thereby producing visual symptoms of Ni deficiency (Brown et al. 1990; Bai et al. 2006a; Wood et al. 2006). However, the amount of Ni normally required by plants is generally very low, and is adequately provided by most soils; hence, deficiency symptoms usually do not appear in plants under natural conditions.

The most important effect of Ni deficiency, when it appears, is on the activity of urease, which catalyzes the conversion of urea to ammonium, and is thus involved in N-assimilation in plants (Spears et al. 1977; Bast 1988; Gerendás and Sattelmacher 1997a; Colpas and Hausinger 2000; Benoit et al. 2007). Plants grown in Ni-deficient nutrient solutions may develop visual symptoms of Ni-deficiency that are generally associated with hampered N-metabolism (Gerendás et al. 1999; Brown 2006; Reddy 2006; Wood et al. 2006). For example, Ni-deficient soybean (*Glycine max* L.) plants were shown to have depressed urease activity in their leaves that resulted in accumulation of urea at toxic levels in leaflet tips (Eskew et al. 1983). Similarly, Walker et al. (1985), working with cowpeas [*Vigna unguiculata* (L.) Walp], suggested that Ni (and urease) participates in N metabolism of legumes during the reproductive phase of growth. Checkai et al. (1986) reported that Ni-deficient tomato plants (*Lycopersicon esculentum* L.) developed chlorosis in young leaves, and ultimately, meristematic necrosis. In some aquatic organisms, nickel is involved in the maintenance of homeostasis, and thus plays an important role in water balance, which is required for optimal growth (Muyssen et al. 2004). Lower levels of nickel are also known to be essential for initiating the reproductive phase and for seed development (Brown 2006; Tabatabaei 2009). Hence, some Ni-deficient plants may fail to produce viable seeds and are unable to complete their life cycle. From the foregoing, it can clearly be seen that Ni is essential for the growth and development of most plants, including some crops.

6 Causes and Symptoms of Nickel Deficiency in Plants

Despite the fact that nickel is essential for normal plant functioning, symptoms of natural deficiency are uncommon because most soils contain adequate amounts of the element (Lindberg and Greger 2002). Moreover, deficiency symptoms produced by Ni are very similar to those produced by deficiencies of other essential plant nutrients (Wood et al. 2004, 2006). For example, Brown (2006) showed that the symptoms of nickel deficiency are very similar to those of zinc and copper, the deficiency of which may arise from complex soil and environmental interactions that influence plant uptake. In addition to soil physicochemical properties (such as pH), nickel deficiency may be induced by excessive concentrations of zinc, iron, manganese, copper, magnesium, or calcium in the soil solution (Wood et al. 2004). Observations also suggest that long-term and excessive use of fertilizers containing

light metals (e.g., Zn, Mg, and Cu) is one primary cause of Ni deficiency (He et al. 2005; Brown 2006). This may be because Cu and Zn inhibit Ni uptake competitively (i.e., these three soluble metal ions are absorbed by the same transport system) (Cataldo et al. 1988; Körner et al. 1987; Kochian 1991). In addition, the soluble compounds of Ni and Mg-ions are absorbed by this same transport system, as both ions have similar charge–size ratio. High application rates of Mg-containing fertilizers may produce Ni-deficiency (Oller et al. 1997). Therefore, it is often difficult to identify whether observed symptoms result from a deficiency of Ni or are caused by a deficiency of certain other metals (Brown 2006; Wood et al. 2006).

The earliest symptoms of Ni deficiency in plants, or plant growth effects in response to the addition of Ni to growth media, under controlled experimental conditions, were reported by Brown et al. (1987a, b). These authors indicated that Ni deficiency has a wide range of effects on plant growth and metabolism, including some prominent ones: (a) reduced vegetative growth (lengths and fresh and dry weights of root and shoot), (b) enhanced plant senescence, (c) changes in N metabolism, and (d) normal Fe uptake. Preliminary investigations also indicate that Ni may have a role in phytoalexin synthesis and in reducing plant disease resistance (Graham et al. 1985).

At lower levels, Ni improves the seed germination of many species. Therefore, under Ni deficit conditions, seeds may experience delayed seed germination (Brown et al. 1987b; Ahmad et al. 2009). Nickel is essential for the proper plant absorption of Fe (Kopittke et al. 2008). Therefore, Ni-deficient plants may develop chlorosis or necrotic spots when the deficiency is extreme (Checkai et al. 1986; Voss 1993; Bennett 1993). When Ni is completely absent, plants show increased flower and fruit loss and reduced crop yield (Brown et al. 1987b; Balaguer et al. 1998). Moreover, Ni-deficient plants display weak and broken branches and poor plant architecture (Wood et al. 2006; Brown 2006).

Other symptoms of Ni deficiency also exist, but they vary by plant species and the extent of the deficiency. Among these symptoms are the following: development of premature leaf chlorosis that may extend to necrosis of entire leaves and/or leaflet tips; blunting of leaf and/or leaflet tips; dwarfing of foliage; curled leaf and/or leaflet margins; failure of lamina to develop properly; reduced internode distance; distortion of bud shape; shoot brittleness; death of overwintering shoots resembling cold injury; resetting and loss of apical dominance; a diminished root system with dead fibrous roots; dwarf trees; and ultimately, death of whole trees or tree parts (Wood et al. 2006).

7 Toxicity of Nickel in Plants

Although Ni is essential to several metabolic phenomena, it is extremely toxic to plants when present at excessive levels in the soil or in nutrient solutions to which plants are exposed. The general signs associated with Ni toxicity in plants include the following: reduced shoot and root growth (Rahman et al. 2005), poor development of

the branching system (Reeves et al. 1996), deformation of various plant parts (Wright and Welbourn 2002), abnormal flower shape (Mishra and Kar 1974; McIlveen and Negusanti 1994), decreased biomass production (Rahman et al. 2005), leaf spotting (Gajewska et al. 2006), mitotic root tip disturbances (McIlveen and Negusanti 1994), inhibition of germination (Nedhi et al. 1990), Fe deficiency leading to chlorosis (Ewais 1997; Kirkby and Römheld 2004), and foliar necrosis (Kukkola et al. 2000). Excess Ni levels also affect nutrient absorption via roots (Kochian 1991; Hasinur et al. 2005), impair plant metabolism (Pandey and Sharma 2002), and inhibit photosynthesis and transpiration (Sheoran et al. 1990). Ultimately, impairment of these processes leads to reduced agricultural crop yields, when excessive Ni levels are present in soils.

8 Effect of Nickel on Seed Germination

Seed germination is one of the most important stages in crop development that preordains establishment of healthy crop stands, better growth and yield at the later growth stages. Germination is strongly influenced by several factors, such as presence or absence of light, germination time, water status, and mineral composition of soil (Mott 1974; Chachalis and Reddy 2000; Zlesak 2007). In addition, environmental stresses such as salinity, drought, and presence of excessive amount of metals in soil also influence the germination ability of plant species (Peralta-Videa et al. 2001; Houlam and Fares 2001; Li et al. 2002; Munzuroglu and Geckil 2002; Zia and Khan 2004).

Although excess Ni, when present, may produce effects at any crop development stage, plant sensitivity to heavy metal or other environmental stress is high at the germination stage (Leon et al. 2005). Increasing concentrations of Ni have been shown to inhibit seed germination and seedling growth in many plant species (Espen et al. 1997; Leon et al. 2005). Ni-induced growth inhibition has been ascribed to downregulation of protein synthesis and to effects on the activities of certain key enzymes responsible for mobilizing food reserves from the endosperm of cotyledons (Foy et al. 1978; Bishnoi et al. 1993a). In addition, Ni is known to be a competitor for several essential micro- and macroelements and may reduce the uptake of elements into germinating seeds, thereby producing poor germination and seedling establishment (Cataldo et al. 1978; Körner et al. 1987; Kochian 1991). The inhibitory effects of high Ni concentrations on seed germination are elaborated on below.

8.1 Germination Percentage and Rate

Nickel, acting as a micronutrient, can improve both the rate and percentage of seed germination. However, high levels of Ni in the growth medium has led to a marked inhibition of germination and has delayed the time necessary to achieve

50% germination (Espen et al. 1997; Leon et al. 2005). Maheshwari and Dubey (2008) reported a significant reduction in the germination of rice seeds, when high concentrations of Ni were present. These authors showed that exposure of seedlings to Ni for 120 h reduced germination by 12–20%, reduced plumule and radicle lengths by 20–53%, and reduced fresh weight by 8–34%. In addition, Ni stress increased the concentration of soluble proteins by 58–101%, and augmented amounts of total free amino acids present in the endosperm and embryo axes by 39–107%. The authors suggested that the high concentration of Ni in the growth medium of rice seedlings imposed stress that resulted in decreased hydrolysis and delayed mobilization of the protein reserves present in endosperm. Hence, high levels of Ni can cause imbalances in the concentration of proteins and amino acids in growing embryo apices. Such effects of high Ni levels may ultimately result in decreased seed germination of most plants.

8.2 Seedling Growth

Although lower Ni levels improve early seed growth, high levels may be extremely toxic and may inhibit seedling growth (Veer 1989; Nedhi et al. 1990; Leon et al. 2005). The reduction in plumule and radicle lengths and reductions in fresh and dry weights were reported for several plant species exposed to high Ni concentrations (e.g., sunflower (Singh et al. 2004, Ahmad et al. 2009), radish (Espen et al. 1997), *Vigna radiata* (Jagetiya and Aery 1998), and tomato (Kowalczyk et al. 2003)). Such high Ni level-induced effects resulted from the inhibition of key enzymes involved in digestion of food reserves (protease and α- and β-amylases), protein synthesis, carbohydrate metabolism, and mobilization of food reserves (Bishnoi et al. 1993a; Lin and Kao 2006; Maheshwari and Dubey 2007). In addition, high Ni levels are known to strongly interfere with mineral nutrient uptake, thereby altering nutrient concentrations in germinating seeds (Gabbrielli et al. 1990; Rubio et al. 1994; Molas 1997; Ahmad et al. 2007). Because micro- and macronutrients are often involved in a variety of metabolic processes, and some are required as cofactors and enzyme activators, any deficiency of them may suppress certain metabolic phenomena involved in regulating seed germination.

8.3 Nutrient Uptake and Transport

The uptake and translocation of plant nutrients during germination and early seedling growth is highly sensitive to the presence of high Ni levels in the growth medium (Cataldo et al. 1978; Gabbrielli et al. 1990). This sensitivity may result from either the direct effect of high Ni on seed imbibition or alterations in the nutrient uptake pattern in germinating seeds (Seregin and Kozhevnikova 2005). However, symptoms of Ni toxicity, caused by Ni stress, may take some time to appear when

Ni interferes with metabolism and causes structural alterations in germinating seeds. Nickel may also interfere with the uptake and transport of essential nutrients, thereby disturbing the status of plant mineral nutrients. For example, concentrations of calcium, magnesium, zinc, iron, manganese, and certain other essential nutrients do change when plants are affected by Ni toxicity (Cataldo et al. 1978; Heale and Ormrod 1982; Körner et al. 1987; Kochian 1991; Marschner 1995; Nieminen and Helmisaari 1996). Therefore, when Ni induces plant stress, the nutrient deficiencies that result may alter seedling growth patterns and produce a poor crop at late crop development stages.

8.4 Amylase and Protease Activities

During germination, many events take place in germinating seeds that involve activation of respiratory enzymes and breakdown and mobilization of food reserves (Bewley and Black 1994; Bewley 1997). This breakdown and mobilization of storage reserves is controlled mainly by α- and β-amylases (both for starch digestion) and protease (protein digestion) (Bishnoi et al. 1993a; Ashraf et al. 2011). These enzymes are activated in germinating seeds soon after imbibition takes place, and they support the respiratory requirements of the actively growing shoot and root apices (Leon et al. 2005; Maheshwari and Dubey 2008). Proper regulation of these enzymes is crucial to ensuring that the balanced supplies of substrates, required for proper metabolic functioning, takes place in the growing embryonic axis (Mayer and Poljakoff-Mayber 1975; Bewley 1997). Therefore, the activity of these enzymes is highly sensitive to stressful conditions, including Ni toxicity. Wang et al. (2001) showed that excess Ni inhibited the mobilization of starch in germinating rice grains and also inhibited starch digestion-associated enzyme activity (α- and β-amylases). Similarly, Maheshwari and Dubey (2007) reported that rice seedlings exposed to high Ni levels in a nutrient medium showed marked decreases in the levels of RNA, total soluble proteins, and total free amino acids. These authors believed that the effects resulted from the reduced activity of ribonuclease (RNase) and protease activities in both roots and shoots.

8.5 Changes in Carbohydrates

Starch is the predominant carbohydrate that is stored in most seeds. Upon imbibition, carbohydrate reserves in the endosperm are mobilized and lead to seed germination and development of the embryonic axis (Briggs 1992). In germinating seeds, carbohydrate metabolism is controlled by many hydrolytic enzymes, including α- and β-amylases, starch phosphorylase, and invertase (acid and alkaline) (Bishnoi et al. 1993a; Yang et al. 2001; Van den Ende et al. 2002). Most toxic metal ions, including Ni, cause a marked perturbation in the metabolism of proteins, carbohydrates, and

nucleic acids, when present in soils at excessive levels (Maheshwari and Dubey 2007). When high levels of Ni is involved, these effects derive from the direct influence on key metabolizing enzymes, which induce alterations in levels of essential biomolecules such as sugars, amino acids, proteins, and nucleotides in germinating seeds and growing plants (Veer 1989). Hence, the concentration of sugars (total, reducing and nonreducing ones) decreases under Ni stress. The effects are direct ones by Ni on the activity of the major hydrolytic enzymes involved in sugar metabolism.

8.6 Proteolytic and Ribonucleolytic Enzymes

Although several enzymes are involved in the reserves mobilization that takes place during seed germination, proteolytic and ribonucleolytic enzymes are also activated during the process, and their activity is important for seedling establishment (Bewley and Black 1994). These enzymes regulate both RNA turnover and mobilization from seed storage tissues (Gomes-Filho et al. 1999). They also exert a major influence on gene expression under certain abiotic and biotic stressful conditions (Booker 2004). In addition to the activity of RNases, the regulation of protein breakdown and recycling is essential for control of seed germination (Palma et al. 2002). Such mobilization of protein reserves is controlled by the enzyme *protease*, which degrades storage proteins and mobilizes amino acids to support the growing embryonic apices of germinating seeds (Yamauchi 2003).

Plants mobilize and utilize biomolecules (e.g., proteins, starch, and nucleic acids), and activate metabolic enzymes in response to stresses (Syros et al. 2005). The activated RNase and protease in germinating seeds affect the protein pool and, therefore, seed germination. The activities of RNase and protease are altered when exposed to high Ni levels (Kirchgessner and Schnegg 1979; Booker 2004). Pena et al. (2006) exposed sunflower plants to various levels of Pb, Al, Ni, Cd, Hg, Co, Cr, and Cu. All of these metals increased protein oxidation levels, although Ni was the most effective in doing so (112%), followed by Cd and Hg (74%) and Co (68%), Cr (64%), Pb (62%), and Al (57%). Copper treatment had the lowest effect on protein oxidation (40% more than the controls). Different metals affect protease activity differently, with Ni being the most toxic in reducing protease activity on sunflower plants. Maheshwari and Dubey (2007, 2008) have recently shown that rice seed germination was significantly reduced when exposed to 200 and 400 μM $NiSO_4$. In addition, Ni stress in germinating seeds produced significantly increased levels of RNA, soluble proteins, and free amino acids in endosperm and embryonic axes. Maheshwari and Dubey (2007, 2008) suggested that the presence of high Ni levels in the medium of the germinating rice seeds induced stress, which decreased hydrolysis and delayed mobilization of endospermic RNA and protein reserves, which, in turn, caused an imbalance in amounts of certain biomolecules (e.g., RNA, proteins, and amino acids) in the growing embryo axes, thereby reducing seed germination.

8.7 Changes in Proteins and Amino Acids

A considerable increase in free amino acid levels occurs in germinating seeds exposed to heavy metals. When such exposure occurs in root growing media, a variety of biochemical and molecular responses are initiated, and these help the plant to tolerate hazardous metal ions. Exposure to Ni caused enhanced accumulation of free amino acids, particularly alanine, proline and asparagine, which is regarded as an indicator of metabolic disruption, in young seedlings of soybean (El-Shintinawy and El-Ansary 2000). These authors reported that Ni stress (200 μM) induced the accumulation of free amino acids in the roots of soybean seedlings. They also reported that cysteine was the most accumulated amino acid (17% of total free amino acids present), when exposed to Ni stress. Maheshwari and Dubey (2008) reported that protease activity decreased and protein concentration increased, when plants were exposed to high Ni regimes. The level of free amino acids in rice seedlings was increased under a high Ni regime, and the authors suggested that free amino acids may be produced in seedlings under Ni stress at the cost of maintenance of developmental processes (Ali and Saradhi 1991). In summary, protein degradation under high Ni regimes may enhance the accumulation of crop plant amino acids, particularly at initial growth stages.

9 Effects of Nickel at the Vegetative Stage

9.1 Growth Attributes

High concentrations of Ni reduce vegetative growth parameters, including plant height and fresh and dry biomass production in several agricultural crops (MacNicol and Beckett 1985; Seregin et al. 2003). As mentioned, the general signs of Ni toxicity in plants include reduced shoot and root growth (Rahman et al. 2005; Sabir et al. 2011), poor branching (Reeves et al. 1996), deformed plant parts (Wright and Welbourn 2002), abnormal flower shape (Mishra and Kar 1974; McIlveen and Negusanti 1994), decreased biomass production (Pandey and Sharma 2002; Rahman et al. 2005), leaf spotting (Gajewska et al. 2006), mitotic root tip disturbances (McIlveen and Negusanti 1994), inhibition of germination (Nedhi et al. 1990), Fe deficiency-induced chlorosis (Ewais 1997; Kirkby and Römheld 2004), and foliar necrosis (Kukkola et al. 2000).

Since roots are directly exposed to excess Ni levels, they may display reduced growth, and proliferation and deformation in shape long before symptoms of toxicity appear in aerial plant parts (Wong and Bradshaw 1982; Yang et al. 1996; Kopittke et al. 2007). For example, a high concentration of Ni is known to inhibit the production of new root hairs and deform existing ones in many plants (e.g., *Betula papyrifera* (paper birch) and *Lonicera tatarica* (honeysuckle); Patterson and Olson 1983; Rauta et al. 1995; Atta-Aly 1999). Similarly, the process of initiation and proliferation

of lateral roots may be inhibited by excess Ni in crops such as maize and rice (Seregin et al. 2003). The reason for such growth inhibition may be that Ni easily crosses the endodermal barrier and accumulates in pericycle cells, thereby affecting cell division and proliferation (Seregin et al. 2003; Seregin and Kozhevnikova 2006).

The Ni-induced toxicity symptoms displayed in many aerial parts of certain plant species start with foliar chlorosis; symptoms first appear primarily in older leaves and gradually move to younger ones (Pandey and Sharma 2002). Sometimes, chlorosis starts at the leaf margins and slowly progresses to the interveinal areas. In other cases, acute Ni toxicity causes necrotic spots to rapidly develop on leaf margins, and then these appear later in the interveinal areas of leaves (Baccouch et al. 1998a). In severe cases, plants may develop necrotic lesions on younger as well as older leaves and eventually cause death of entire leaves (Sun and Wu 1998). Since Ni strongly competes with other essential elements, (such as Mg, Fe, Cu, Zn, and Mn), the typical visual symptoms of Ni toxicity result from a deficiency of any of these essential nutrients (Khalid and Tinsley 1980; Hasinur et al. 2005).

Many mechanisms have been proposed to explain how Ni produces its effects on plant growth and development. Proposals for causative mechanisms include (1) a general metabolic disorder (Mishra and Kar 1974; Baccouch et al. 1998a; Murch et al. 2003), (2) a decrease in cell wall plasticity (Pandolfini et al. 1992), (3) impaired cell division and elongation (Robertson and Meakin 1980; Demchenko et al. 2005), (4) disorganization of nuclear structures (Sresty and Rao 1999), (5) chromosomal aberrations (Liu et al. 1995), and/or nutritional imbalances (Ahmad et al. 2011). The injury produced by treatment with 1.5–5.00 mM $NiCl_2$ caused mitotic tip arrest of *Vicia faba* roots. Similarly, Demchenko et al. (2005) reported that treatment of embryonic wheat (*Triticum aestivum*) roots with 0.1 mM $NiSO_4$ blocked cell division in all except the distal ends of rhizodermal, exodermal, and middle curricular areas, and in the peripheral cells of the caliptrogen. Sresty and Rao (1999) concluded that inhibition of cell division often results from disruption of the nuclear membrane, which causes nuclear structure disorganization (e.g., development of two nucleoli in the nucleus and extensive condensation of chromatin). Regardless of the exact mechanism by which Ni induces plant toxicity, ultimately, Ni-induced stress reduces vegetative growth and decreases yield (Balaguer et al. 1998; Tabatabaei 2009).

9.2 Membrane Permeability and Electrolyte Leakage

Compared to other known abiotic stresses, a few reports exist that address the effect of metal ions on biological membrane permeability. However, it is known that ion leakage is an important consequence of metal stress in plants from exposure to Cu and Ni (Rao and Sresty 2000; Seregin and Kozhevnikova 2006). Membrane permeability may be damaged due to high Cd and Ni exposure levels, and contact with these metals induces loss of key osmolytes and turgor pressure (Wang et al. 2008; Llamas et al. 2008).

Excessive amounts of reactive oxygen species (ROS) are produced from exposure to Ni in plants (Gajewska et al. 2006; Gajewska and Skłodowska 2007). ROS produce oxidative damage to membrane lipids and proteins from lipid peroxidation and membrane degradation (Dat et al. 2000; Verma and Dubey 2003). Plants defend themselves from such oxidative attacks by enzymatic means. Examples of such defense enzymes are catalase (CAT), peroxidase (POD), superoxide dismutase (SOD), glutathione reductase, and others. In addition, plants use nonenzymatic means to defend themselves, most notably by exuding compounds that are antioxidants, to wit, ascorbic acid (AsA), phenolics, tocopherols, and reduced glutathione (GSH) antioxidants. These natural entities help plants to alleviate the toxic effects of ROS by removing, neutralizing, or scavenging them (Pandolfini et al. 1992; Noctor and Foyer 1998; Alscher et al. 2002; Verma and Dubey 2003; Freeman et al. 2004; Maheshwari and Dubey 2009). However, when high exposure levels to metals occur, these defenses often fail to sufficiently scavenge excess ROS; the result is enhanced lipid peroxidation and electrolyte leakage (Howlett and Avery 1997; Zhang et al. 2007; Wang et al. 2008). Because Ni is a redoxically inactive metal, it does not directly generate ROS (Boominathan and Doran 2002). However, in some previous studies, it has been reported that excessive ROS generation takes place under high Ni application that causes membrane lipid peroxidation (Baccouch et al. 1998b; Rao and Sresty 2000; Gajewska and Skłodowska 2005) and decreased protein thiolation, in many plant species (Maheshwari and Dubey 2009). Therefore, such changes in sterol and phospholipid composition of the plasma membranes may occur at high Ni levels that might alter plant membrane permeability functions (Ros et al. 1990).

As mentioned previously, Ni is known to compete with several other essential metal nutrients (e.g., Mg, Mn, Fe, Zn, and Ca; Heale and Ormrod 1982; Marschner 1995; Cataldo et al. 1978; Körner et al. 1987; Kochian 1991; Küpper et al. 1996). Therefore, when Ni is present in plant tissues, the concentration of these other nutrients tend to decrease (Gabbrielli et al. 1990; Rubio et al. 1994; Rahman et al. 2005; Ahmad et al. 2007). Ca and Zn are required for membrane stability (Valko et al. 2005; Taiz and Zeiger 2006) and Fe is a constituent (cofactor) of many antioxidative enzymes (e.g., catalase and peroxidase; Ranieri et al. 2003). Therefore, a Ni-induced Fe-deficiency may decrease the activities of antioxidant enzymes, and hence, increase lipid peroxidation, with a consequential membrane permeability loss (Baccouch et al. 1998b, 2001; Boominathan and Doran 2002).

9.3 Photosynthetic Pigments

High Ni levels in plants may alter the concentrations of photosynthetic pigments, such as chlorophyll *a*, *b* and carotenoids (McIlveen and Negusanti 1994; Balaguer et al. 1998; Seregin and Kozhevnikova 2006). These changes commonly induce leaf chlorosis and necrosis, which are common symptoms of Ni toxicity in plants (Gajewska et al. 2006). Excess Ni concentrations may directly damage the photosynthetic apparatus of leaves in several ways. Excess Ni may destroy mesophyll and

epidermal cells (Heath et al. 1997; Hermle et al. 2007), deteriorate grana structure and thylakoid membranes of chloroplasts (Szalontai et al. 1999; Molas 2002), decrease grana size and increase the number of lamellae in nonappressed regions (Molas 1997). Such modifications reduce chlorophyll (*a*, *b*, total), xanthophylls, and carotenoids (Krupa et al. 1993; Pandey and Sharma 2002; Gajewska et al. 2006; Ahmad et al. 2007).

Nickel also competes with other essential nutrients in plants for uptake and translocation, and may reduce their concentrations to levels of deficiency. Therefore, under Ni stress, the concentrations of Fe, Cu, Zn, Mg, Fe, and Mn may decrease (Krupa and Baszynski 1995). This can produce secondary effects (Ewais 1997; Gajewska et al. 2006; Shukla and Gopal 2009), because Mg is an integral part of the chlorophyll and heme structures. Moreover, Fe and Mn are required for proper chlorophyll metabolic functioning. When Ni toxicity is extreme, the chlorophyll in chloroplasts may break down completely, producing leaf chlorosis and necrosis.

9.4 Photosynthesis

Nickel inhibits plant photosynthesis and alters gas exchange in plants (Nedhi et al. 1990; Bishnoi et al. 1993b; Krupa et al. 1993). This may reduce the net photosynthetic rate (Sheoran et al. 1990; Bishnoi et al. 1993b; Krupa and Baszynski 1995), stomatal conductance (Heath et al. 1997), transpiration rate (Bishnoi et al. 1993b; Pandey and Sharma 2002), and water-use efficiency (Bishnoi et al. 1993b), when toxic levels of Ni are present in plants. Toxic effects are also produced when Ni is applied either directly to isolated chloroplasts of guard cells, or through roots (Tripathy et al. 1981; Singh et al. 1989; Molas 2002; Boisvert et al. 2007). These findings indicate that Ni may indirectly affect stomatal opening through alteration in K^+ fluxes across guard cell membranes. The reduced size of the stomatal aperture results in decreased gaseous exchange across leaf surfaces that leads to reduced photosynthetic rates (Nedhi et al. 1990; Bishnoi et al. 1993b). However, in other studies, a stable or increased transpiration rate and stomatal conductance, but a decreased net photosynthetic rate, have been reported. In this case, the reduction in photosynthetic rate appears to be a result of the toxic effects of Ni on metabolically important phenomena, rather than on stomatal regulation (Moya 1995; Malkin and Niyogi 2000; Papazogloua et al. 2007).

Excess Ni may also nonspecifically inhibit photosynthesis, and thereby damage chloroplast structure (Heath et al. 1997; Hermle et al. 2007), reduce chlorophyll synthesis or enhance its breakdown (Seregin and Kozhevnikova 2006), disorder electron transport (Singh et al. 1989; Tripathy et al. 1981), inhibit Calvin cycle enzymes, and affect stomatal closure, thereby inducing a CO_2 deficit in chloroplasts (Sheoran et al. 1990). Molas (1997) reported that Ni-stressed *Brassica oleracea* plants (10–20 mg L^{-1}) suffered reduced chloroplast size, disorganized and deformed grana and thylakoid membranes, and altered lipid composition of chloroplast membranes. The authors suggested that these Ni-induced changes may arise from

decreased cell moisture content or increased oxidative stress, resulting in peroxidation of membrane lipids. Other authors have reported similar chloroplast structural and functional changes in the leaves of Ni-treated plants; such changes ultimately produce chlorosis and leaf necrosis (Khalid and Tinsley 1980; Ewais 1997; Sheoran et al. 1990; Piccini and Malavolta 1992).

Nickel may also reduce the photosynthetic rate by inhibiting components of the light and dark reactions. Nickel is known to disrupt electron transport during the light reaction (Krupa and Baszynski 1995; Malkin and Niyogi 2000). Photosystem II (PSII) is the primary site of Ni-induced electron transport chain (ETC) inhibition, and in that regard is similar to how other metals act (Mohanty et al. 1989; Krupa and Baszynski 1995). Veeranjaneyulu and Das (1982) showed that the predominant sites of Ni deposition are in lamellar regions of chloroplasts that contain PSII. In addition, Ni also reduces cyt. b_6-f and b_{559}, ferredoxin, and plastocyanin in thylakoid membranes. Moreover, Ni may inhibit photosynthesis by affecting key enzymes involved in the operation and regulation of the Calvin cycle (dark reaction). Key enzymes of the dark reaction that are affected by metals, other than Ni, include the following: rubisco, 3-PGA kinase, F-1,6-bisphosphatase, aldolase, and phosphoglyceraldehyde dehydrogenase (both NAD- and NADP-dependent) (Sheoran et al. 1990). Inhibition of these dark reaction enzymes leads to the accumulation of light-reaction products, such as ATP and NADPH. This, in turn, produces a high pH gradient across the thylakoid membrane, which blocks PS-II activity (Krupa and Baszynski 1995).

In summary, metal effects on metabolic processes during the light and dark reactions may cause direct inhibition of photosynthesis. These metabolic alterations inhibit plant growth and disrupt morphogenesis. Notwithstanding, the disruption of photosynthesis by Ni cannot be attributed to any single factor, and appears to derive from a combination of factors that disrupt chloroplast structure and function, chlorophyll content and photosynthetic protein complex functioning.

9.5 Water Relations

Plant water relations are among the most important parameters that are affected by environmental stresses (Kramer and Boyer 1995). However, whether they are affected by heavy metal stresses is unclear, as there are contrasting reports in the literature on the effects of metal stress on plant water relations (Barcelo and Poschenrieder 1990; Menon et al. 2005; Vernay et al. 2007). Furthermore, little research has been conducted on the effects of heavy metals on plant water relations, and most such work has addressed other stressors, such as drought, salinity and temperature. Still, many authors believe that there is evidence to support the view that metal stress does affect imbalances in plant water relations (Sharma and Sharma 1987; Barcelo and Poschenrieder 1990; Chatterjee and Chatterjee 2000). Unfortunately, this topic is fraught with considerable complexity and different opinions exist that vary with the type of metal, the concentration of the metal studied, plant species or genotypes tested, exposure time, etc. (Kastori et al. 1992)

Rauser and Dumbroff (1981) examined water relations in bean seedlings exposed to excess Ni and Co. These authors reported no significant change in the relative water content (RWC) of bean leaves during early periods of metal stress. However, RWC decreased significantly during later periods of stress. These authors also reported that metal-treated plants had a significant increase in leaf water potential of unifoliate leaves on the first day the metal was applied. However, on the second day, the water potentials in leaves treated with Co and Zn returned to the levels observed in the controls, whereas leaves in the Ni treatment showed a steep decline in water potential values.

When Ni stress is present, plant water relations may undergo changes, possibly from the accumulation of compatible solutes/osmotica. For example, proline accumulates in plants under Ni stress (Ali and Saradhi 1991; Mehta and Gaur 1999; El-Enany and Issa 2001; Lin and Kao 2007). In addition, other osmotica, such as soluble sugars, and other free amino acids, also accumulate in Ni-stressed plants (Baccouch et al. 1998a). These solutes can significantly reduce the water and solute potentials in Ni-stressed plants. Sharma and Dietz (2006) believe that the proline effects on osmoregulation under Ni stress are obvious, but the effects of other osmotica (e.g., sugars, amino acids, and proteins), are less clear.

9.6 Free Amino Acids

In metal stressed environments, free amino acids may accumulate in cultivated crops. The accumulation results in a decrease in the total protein concentration (Ali et al. 2009). Various amino acids that include alanine (Bhatia et al. 2005), arginine (Ali et al. 2009), asparagine (Smirnoff and Stewart 1987), glycine (Bhatia et al. 2005; Ali et al. 2009), cysteine (Freeman et al. 2004; Ali et al. 2009), glutamic acid (Homer et al. 1997), histidine (Krämer et al. 1996; Ali et al. 2009), lysine (Ali et al. 2009), methionine (Ali et al. 2009), proline (Gajewska et al. 2006; Maheshwari and Dubey 2007; Gajewska and Skłodowska 2005; 2008), and serine (Ali et al. 2009), are known to accumulate at high concentrations, when metal (i.e., Ni, Zn, Cr, Cd, Pb) concentrations are high. Some amino acids, such as proline, function as protectants, while others, such as asparagine, function as chelating agents that form metal–ligand complexes with Cd, Pb, Ni, Zn, etc. (Homer et al. 1997). High accumulations of free amino acids may, therefore, protect stressed plants from the toxic effects of specific metals through a detoxification mechanism (Clemens 2001; Hall 2002).

The accumulation of an amino acid in response to heavy metal exposure is a species-specific trait that varies greatly among species, particularly in hyperaccumulator or metal-tolerant plants. White et al. (1981) reported that the major portion of Cu and Ni was bound to asparagine and histidine in xylem fluids. Smith and Martell (1989) suggested that, among all the organic acids and proteinacious amino acids, histidine has the highest association constant for complexation with Ni. Harmens et al. (1993) reported that cysteine titers increased in tolerant and sensitive ecotypes of *Silene vulgaris* by factors of 4.5 and 3.8, respectively, in response to

metal stress. Homer et al. (1997) determined the accumulation pattern of amino acids in three species of Ni-hyperaccumulator plants (*Dechampetalum geloniodes, Phyllanthus palwanensis, and Walsura monophylla),* and reported that, with one exception, proline was the most abundant amino acid among all those studied; the exception was for *W. monophylla*, which accumulated glutamine in higher quantity than it did proline. Bhatia et al. (2005) studied the changes in the amino-acid profile that occurred in the xylem sap of *Stackhousia tryonii* (Ni-hyperaccumulator). They reported a slight decrease in the concentration of total amino acids under Ni stress, which contrasted with earlier observations. The proportion of glycine declined considerably from 48 to 22%. However, that of alanine, asparagine, and glutamine increased under Ni stress. The authors suggested that asparagine and alanine may contribute to Ni complexation in the xylem of hyperaccumulator species (Bhatia et al. 2005). Freeman et al. (2004) reported that the accumulation of *o*-acetyl-L-serine, cysteine and glutathione are strongly correlated with Ni accumulation and tolerance ability in various *Thlaspi* hyperaccumulator plants. They concluded that elevated levels of these amino acids play a causal role in Ni tolerance by enhancing GSH-dependent antioxidant activity. Moreover, Ali et al. (2009) suggested that Ni, although absorbed by roots as the free cation under control conditions, changes under Ni stress conditions. Under Ni stress some canola cultivars synthesized high amounts of amino acids (i.e., histidine and cysteine), which may have enhanced Ni translocation from root to shoot and accentuated detoxification (Hall 2002).

The foregoing results indicate that complexation between amino acids and Ni is more stable than complexation with carboxylic acids (Krämer et al. 1996; Homer et al. 1997; Kerkeb and Krämer 2003). Moreover, the accumulation of amino acids under metal stress conditions is one of the most important detoxification mechanisms, particularly in hyperaccumulator plants (Clemens 2001; Hall 2002).

9.7 Proline

The accumulation of free proline under Ni stress has been reported in several studies. The protective functions of proline have been attributed to its roles as an osmoprotectant (Paleg et al. 1984), osmo- and redox-regulator (Sharma and Dietz 2006), membrane stabilizer (Bandurska 2001; Matysik et al. 2002), metal chelator (Cobbett 2000), ROS scavenger (Alia et al. 2001), and enzyme protector (Sharma and Dubey 2007). For example, an eightfold increase in the free proline content of Ni-treated *Chlorella vulgaris* was observed by Mehta and Gaur (1999). Gajewska and Skłodowska (2005; 2008) reported that Ni stressed pea and wheat plants accumulated high quantities of proline that significantly reduced oxidative damage to membranes and proteins. Similarly, Ghorbanli et al. (2006) reported that Ni treated *Brassica* plants accumulated more proline than did normal plants. From these reports it is inferred that proline accumulation augments plant tolerance to Ni stress, and further, could be used as an important metabolic indicator of Ni tolerance. However, because there are so few data, it is difficult to affirm that the enhanced accumulation of proline in plants is an adaptive response to Ni-induced stress.

9.8 Enzymatic and Nonenzymatic Antioxidants

Nickel is known to be a redox-inactive metal. Therefore, it cannot directly generate ROS (Boominathan and Doran 2002). However, many nonenzymatic antioxidants such as GSH and AsA (Freeman et al. 2004) and enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione *S*-transferase (GST) have been shown to be stimulated by Ni stress (Gabbrielli et al. 1999; Rao and Sresty 2000; Baccouch et al. 2001; Gajewska and Skłodowska 2008). For example, Gajewska and Skłodowska (2007) reported a 250% increase in the concentration of free radicals, such as O_2^- and H_2O_2, in the leaves of Ni-stressed wheat plants after a 3-day exposure to externally applied Ni. They observed a decrease in antioxidative enzymes, such as SOD and CAT, from Ni stress, but observed a significant increase in APX and POD activities at the same exposure. However, lipid peroxidation in leaf tissue membranes remained unchanged after application of Ni. This indicates that APX and POD may have efficiently removed ROS, thereby preventing lipid peroxidation of biomembranes. Similar results were presented by Gajewska et al. (2006); these authors found decreased SOD and CAT activities, but increased POD and GST under conditions of increased Ni stress. Randhawa et al. (2001) experienced contrasting results while working with the green alga *Scenedesmus acutus*. These authors reported an increase in GSH, CAT, and SOD activities under Ni stress. Wang et al. (2010) also reported a significant increase in the activity of CAT, GPX, PAL, and SOD in cotyledons, stems, and roots of Ni-stressed *Luffa cylindrica* seedlings. It is now well known that SOD catalyzes disproportionation of O_2^- to H_2O_2 and O_2, and hence is considered to be a first line of defense against ROS. Harmful concentrations of H_2O_2 are scavenged by catalase (CAT). CAT converts H_2O_2 to H_2O and O_2, whereas the alternative scavenger, ascorbate peroxidase (APX) catalyzes a reduction of H_2O_2 using ascorbate as an electron donor. Other peroxidases like POD are also capable of eliminating H_2O_2 by oxidizing phenolic compounds at the expense of H_2O_2. These enzymes constitute a second line of defense against the ROS production (Gaspar et al. 1991). The results of the foregoing papers make it clear that the antioxidative enzymatic system is stimulated under Ni stress and plays a key role in helping crop plants tolerate excessive Ni levels.

9.9 Soluble Proteins

Heavy metals reduce total soluble protein levels in many plant species (Kastori et al. 1992). In the presence of Ni stress, this reduction generally results from reduced synthesis or accelerated hydrolysis of proteins. Kevresan et al. (1998) reported sugar beet as experiencing a significant decrease in protein content when exposed to Cd and Ni. However, occasionally, soluble protein levels may increase under metal stress. Ewais (1997) reported increased protein content in roots and shoots of *Cyperus difformis* after exposure to Cd, Ni, and Pb.

Most heavy metals inhibit protein activity by altering their structure (Lee et al. 2002a). Therefore, decreased protein content is a sign of metal toxicity to crop plants (Seregin and Kozhevnikova 2006). Reduced titers of plant proteins may occur through several mechanisms. First, Ni may produce oxidative stress, by enhancing ROS production, which, in turn, causes direct protein damage (Baccouch et al. 1998b, 2001; Gajewska et al. 2006). Second, Ni may deplete the abundance of low-molecular-weight proteins, and, thereby, induce oxidative stress (Rao and Sresty 2000; Kukkola et al. 2000). Third, heavy metals may bind to functional groups, mainly protein SH-groups, and thereby modify protein structure. This last mechanism may reduce the activities of enzymes that contain SH-groups (Seregin and Kozhevnikova 2006).

Proteins, polypeptides and ligands play a vital role in the tolerance of plants to Ni (Kim et al. 2005; Seregin and Kozhevnikova 2006). First, such entities bind Ni to N-, O-, or S-ligands (Van Assche and Clijsters 1990; Clemens 2001; Vacchina et al. 2003; Montarges-Pelletier et al. 2008). Second, Ni binds to polypeptides such as phytochelatins (Kim et al. 2005), which play a crucial role in heavy metal tolerance of plants (Steffens 1990; Cobbett 2000; Vacchina et al. 2003). Third, some proteins are able to complex with Ni, which mitigates its toxic effects to plants. Examples of such complexing agents are permeases (Wolfram et al. 1995; Eitinger and Mandrand-Berthelot 2000), metallothioneins (MT) (Schor-Fumbarov et al. 2005), and metallochaperones (Hausinger 1997; Olson et al. 1997; Watt and Ludden 1998).

9.10 Nutrient Accumulation

High concentrations of Ni in a plant growth medium interfere with the uptake of many essential macro- and micro-nutrients (Kochian 1991; Hasinur et al. 2005). As mentioned above, when plants are stressed by the presence of excessive amount of Ni, they may take up reduced amounts of N, P, K, and S (Singh 1984; Dahiya et al. 1993; Aziz et al. 2007; Ali et al. 2009). Similarly, Palacios et al. (1998) reported that Ni stress can significantly reduce the absorption and translocation of Na in plant tissues. Furthermore, plant-leaf photosynthesis studies have shown that Ni competitively removes Ca ions from its binding site in the oxygen evolving complex (Boisvert et al. 2007) and replaces the Mg ion in chlorophyll pigment (Küpper et al. 1996). When this occurs, PSII electron transport is eventually inhibited, which reduces the available energy supply for nutrient uptake. Reduced nutrient uptake may result, thereby leading to nutrient deficiency in plants tissues (Khalid and Tinsley 1980; Moya 1995; Ahmad et al. 2007; Liu 2008).

When Ni competitively mitigates the absorption of certain other micronutrients (e.g., Mn, Fe, etc.), selective micronutrient deficits may occur in plant tissues. Therefore, when excessive Ni levels exist, plants are likely to show symptoms of nutrient deficiency (Gabbrielli et al. 1990; Rubio et al. 1994; Rahman et al. 2005;

Ahmad et al. 2007). Such selective nutrient deficiency may retard metabolic processes and ultimately produce Ni toxicity in plants (Genrich et al. 1998; Gajewska et al. 2006; Gonçalves et al. 2007). Nickel may also suppress growth and development and reduce agricultural crop yields (Nedhi et al. 1990; Rao and Sresty 2000; Boominathan and Doran 2002; Seregin and Kozhevnikova 2006). In addition, some metals (e.g., Fe, Cu, Zn, and Mn) are integral to certain metalloenzymes such as superoxide dismutase (SOD) and catalase (CAT). Therefore, if Ni competes with or otherwise affects their presence, then reduced biosynthesis may result (Molas 2002; Gajewska et al. 2006).

10 Yield and Yield Components

High Ni levels are known to reduce crop yields. Reduced yields in the presence of Ni has been documented to have occurred in: mungbean (Ahmad et al. 2007), tomato (Balaguer et al. 1998), cucumber (Aziz et al. 2007; Tabatabaei 2009), and sunflower (Lavado 2006). Nickel-induced crop yield decreases usually result from impaired root absorption of nutrients (Kochian 1991; Hasinur et al. 2005), impaired plant metabolism (Pandey and Sharma 2002), and/or a decline in photosynthesis and transpiration rates (Sheoran et al. 1990; Shi and Cai 2008). Matraszek et al. (2002) studied the effect of different Ni levels on the yield of vegetables (i.e., spinach, lettuce, and *Phaseolus vulgaris)*. Their results showed that the yield of usable plant parts was significantly reduced, even at very low Ni concentrations (10 mg L^{-1}). Similarly, Singh and Nayyar (2001) reported that dry matter production in cowpea was reduced at soil concentrations of 50 mg Ni kg^{-1} and above. They further showed that cowpea tolerates relatively higher amounts of Ni in the soil, than does other crops. By contrast, lower Ni levels (i.e., 50 mg kg^{-1} soil) strongly improved yields of some plant species (Atta-Aly 1999).

Nickel negatively affects the length and fresh weight of stems, branches and the leaf fresh weight of many plants. It also reduces flowers and fruits of several plant species (Balaguer et al. 1998; Mizuno et al. 2003). An example of Ni toxicity appeared in four cultivars of blackgram (*Vigna mungo*), grown in sandy loam soil (pH 6.3) and amended with 0, 50, 100, 150, or 200 mg Ni kg^{-1}; these plants displayed a reduction in the following: root and shoot lengths, dry matter yields, number of root nodules, and leaf area (Chawan 1995).

Root length, plant weight, leaf area, and seed yield in wheat (*Triticum aestivum*) were significantly affected by Ni exposures from 0 to 1,000 μg L^{-1}. A maximum spike length was observed at 100 μg Ni L^{-1}; however, at 25 μg L^{-1} of Ni, seed weight per 100 seeds was at the maximum (Yadav and Aery 2002; Keeling et al. 2003). Ultimately, crop yield depends on the interplay among a multitude metabolic processes that occur at various plant life cycle stages. These processes result in a yield of seed or biomass, or both, and the foregoing results indicate that the presence of Ni may affect such yield.

11 Anatomical Changes

In addition to growth effects, heavy metal exposure may produce more local effects on certain plant structures. An example is in the leaves of *Triticum aestivum,* when the plant is exposed to a 1 mM $NiSO_4$ solution. After exposure, this plant showed decreases in the following structures: thickness of mesophyll cells, size of vascular bundles, diameter of vessels present in the main and lateral vascular bundles, and width of epidermal cells (Kovacevi et al. 1999). Similarly, the volumes of intercellular spaces, palisade and spongy mesophylls in *Brassica oleracea* leaves decreased, when grown in agar with 10–20 g m^{-3} $NiSO_4.7H_2O$ (Molas 1997). Kravkina (2000) found that Ni-exposed *Dianthus repens* plants formed large leaf inclusions in mesophyll and bundle sheath cells. These were thought to result from complexation of Ni with proteins.

Molas (1997) reported that Ni-stressed cabbage plants had a reduced number of stomata per unit area, as well as fewer open stomata in leaves. In addition, Ni deformed the stomata and increased the number of defective stomata in both adaxial and abaxial leaf surfaces of cabbage plants. Nickel-treatment reduced the volume of spongy and palisade mesophyll cells in comparison with controls in all tested plants. However, the number of mesophyll cells on the same leaf cross sections was simultaneously increased. Compared to controls, the intercellular spaces of mesophyll tissue increased at a low Ni concentration (5 mg L^{-1}), but decreased at a high concentration (10 and 20 mg L^{-1}). In 5 mg L^{-1}-treated plants, the number of chloroplasts in mesophyll cells was higher than that in the control. A reduction of grana size and an increase in the number of nonappressed lamellae of chloroplasts were also observed to occur in cabbage plants. These anatomical modifications were accompanied by overall reduction in leaf chlorophyll content, indicating a reduction in the functionality of light harvesting complexes of the leaf photosynthetic apparatus.

In Ni-treated *Thlaspi japonicum* plants grown on ultramafic soil, the Ni content was highest (3,424 mg kg^{-1}) in the lower epidermis, which also had numerous stomata. Leaf edges and upper epidermis had the second highest Ni content, but these had few stomata. The lowest Ni content occurred in mesophyll cells. Using a microscope, dimethylglyoxime-staining disclosed a Ni-compound presenting as rod-shaped crystals, and these appeared mainly in areas around stomata and projections of the leaf edge. In addition, a considerable amount of Ni was found to be excreted via the guttation fluids (Mizuno et al. 2003)

Heavy metals have been shown to alter stem tissue organization in some crop plants. In particular, Ni caused disorganization of epidermal cells and distortion and disintegration of root cortical cells of wheat and pigeon pea (Setia and Bala 1994; Sresty and Rao 1999). Nickel may also reduce stem diameter, number of vascular bundles and cell size in plant storage tissues. Moreover, exposure to Ni decreases cell wall thickness in stem epidermal and hypodermal tissues (Setia and Bala 1994), and decreases stem and root diameter in wheat cortical cells, when grown in sand culture.

The alteration in width and thickness of leaf midribs and the diameter of xylem vessels of root, stem and leaves were observed in plants subjected to high Ni

concentrations. High Ni exposure decreased stem parenchymatous cell area, leaf midrib tissue, and pith-cortex of roots in wheat. Dimensions of stem vascular bundles were also affected, and root xylem vessels were reduced in number, as was the density of stomata on abaxial leaf surfaces. Such reduced cell size in stems, roots and leaves may be a direct effect of metal-induced inhibition of cell elongation (Kovacevi et al. 1999).

12 Conclusions

Nickel, at low concentrations, acts as a micronutrient and effectively enhances the growth of many crop plants. It has many essential roles in plants: it is a constituent of many metalloenzymes such as urease, some Ni-containing superoside-dismutases (SOD), NiFe hydrogenases, methyl coenzyme M reductase, carbon monoxide dehydrogenase, acetyl coenzyme-A synthase, hydrogenases, and RNase-A. Therefore, Ni deficiencies result in reduced urease activity and perturbance of N assimilation and SOD activity, thereby reducing scavenging of superoxide free-radical. Another important role for Ni in plants is its contribution to phytoalexin synthesis, and hence plant stress resistance. In bacteria and fungi, Ni participates in important metabolic reactions such as hydrogen metabolism, methane biogenesis, and acetogenesis. Therefore, Ni deficiency produces several plant growth symptoms and metabolic effects that pertain to senescence, N metabolism, and Fe uptake. Ni-deficient plants may develop chlorosis in the newly emerged leaves, thereby causing meristematic necrosis.

Most soils are not Ni-deficient. However, Ni often causes plant toxicity. When affected by Ni exposure, plants show reduced shoot and root growth, poor branching development, deformed structures abnormal flower shape, decreased biomass production, leaf spotting, mitotic root tip disturbances, inhibition of germination, and induction of Fe deficiency leading to chlorosis and foliar necrosis. Other Ni toxicity symptoms include reduced nutrient absorption by roots, and decreases in root development, and metabolism, along with photosynthesis and transpiration inhibition. Nickel can replace Co and certain other heavy metals at metalloenzyme active sites and disrupt their functioning. All of these toxic effects reduce agricultural crop yields.

The main toxic effects caused by Ni exposure affect germination, growth, photosynthesis, nutrient accumulation, and crop yield. However, little research has been conducted on changes to membrane permeability, water relation parameters, and patterns of Ni-induced accumulation of solutes (e.g., proline). All of these topics are ones that require further research investigation. In addition, not one report exists in the literature on how Ni affects glycinebetaine, or how it potentially may alter plant growth regulators. These also constitute fertile ground for new research work. Although Ni-induced modifications in the cellular structure of leaves, stems, and roots are well known, new research is needed to further explore whether such modifications confer Ni tolerance to plants. New research in these areas promises to improve the mechanistic understanding of how plants tolerate Ni toxicity.

13 Summary

With the world's ever increasing human population, the issues related to environmental degradation of toxicant chemicals are becoming more serious. Humans have accelerated the emission to the environment of many organic and inorganic pollutants such as pesticides, salts, petroleum products, acids, heavy metals, etc. Among different environmental heavy-metal pollutants, Ni has gained considerable attention in recent years, because of its rapidly increasing concentrations in soil, air, and water in different parts of the world.

The main mechanisms by which Ni is taken up by plants are passive diffusion and active transport. Soluble Ni compounds are preferably absorbed by plants passively, through a cation transport system; chelated Ni compounds are taken up through secondary, active-transport-mediated means, using transport proteins such as permeases. Insoluble Ni compounds primarily enter plant root cells through endocytosis. Once absorbed by roots, Ni is easily transported to shoots via the xylem through the transpiration stream and can accumulate in neonatal parts such as buds, fruits, and seeds. The Ni transport and retranslocation processes are strongly regulated by metal–ligand complexes (such as nicotianamine, histidine, and organic acids) and by some proteins that specifically bind and transport Ni.

Nickel, in low concentrations, fulfills a variety of essential roles in plants, bacteria, and fungi. Therefore, Ni deficiency produces an array of effects on growth and metabolism of plants, including reduced growth, and induction of senescence, leaf and meristem chlorosis, alterations in N metabolism, and reduced Fe uptake. In addition, Ni is a constituent of several metallo-enzymes such as *urease, superoxide dismutase, NiFe hydrogenases, methyl coenzyme M reductase, carbon monoxide dehydrogenase, acetyl coenzyme-A synthase, hydrogenases,* and *RNase-A*. Therefore, Ni deficiencies in plants reduce urease activity, disturb N assimilation, and reduce scavenging of superoxide free radical. In bacteria, Ni participates in several important metabolic reactions such as hydrogen metabolism, methane biogenesis, and acetogenesis.

Although Ni is metabolically important in plants, it is toxic to most plant species when present at excessive amounts in soil and in nutrient solution. High Ni concentrations in growth media severely retards seed germinability of many crops. This effect of Ni is a direct one on the activities of amylases, proteases, and ribonucleases, thereby affecting the digestion and mobilization of food reserves in germinating seeds. At vegetative stages, high Ni concentrations retard shoot and root growth, affect branching development, deform various plant parts, produce abnormal flower shape, decrease biomass production, induce leaf spotting, disturb mitotic root tips, and produce Fe deficiency that leads to chlorosis and foliar necrosis. Additionally, excess Ni also affects nutrient absorption by roots, impairs plant metabolism, inhibits photosynthesis and transpiration, and causes ultrastructural modifications. Ultimately, all of these altered processes produce reduced yields of agricultural crops when such crops encounter excessive Ni exposures.

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References

Abbanat DR, Ferry JG (1991) Resolution of component proteins in an enzyme complex from *Methanosarcina thermophila* catalyzing the synthesis or cleavage of acetyl-CoA. PNAS: 88:3272–3276.

Ahmad MSA, Ashraf M, Hussain M, Ozturk M (2011) Effect of nickel on yield and levels of macro- and micro-nutrients in sunflower (*Helianthus annuus* L.) achenes. J Hazard Mat 185:1295–1303.

Ahmad MSA, Hussain M, Ashraf M, Ahmad R, Ashraf MY (2009) Effect of nickel on seed germinability of some elite sunflower (*Helianthus annuus* L.) cultivars. Pak J Bot 41:1871–1882.

Ahmad MSA, Hussain M, Saddiq R, Alvi AK (2007) Mungbean: A nickel indicator, accumulator or excluder? Bull Environ Contam Toxicol 78:319–324.

Ali AP, Saradhi PP (1991) Proline accumulation under heavy metal stress. J Plant Physiol 138:504–508.

Ali MA, Ashraf M, Athar HR (2009) Influence of nickel stress on growth and some important physiological/biochemical attributes in some diverse canola (*Brassica napus* L.) cultivars. J Hazard Mat 172: 964–969.

Alia, Mohanty P, Matysik J (2001) Effect of proline on the production of singlet oxygen. Amino Acids 21:195–200.

Alloway BJ (1995) Heavy Metals in Soils. Springer, The Netherlands.

Al-Mjeni F, Ju T, Pochapsky TC, Maroney MJ (2002) XAS investigation of the structure and function of Ni in acireductone dioxygenase. Biochemistry 41:6761–6769.

Alscher RG, Erturk N, Heath LS (2002) Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. J Exp Bot 53:1331–1341.

Antoniadis V, Robinson JS, Alloway BJ (2008) Effects of short-term pH fluctuations on cadmium, nickel, lead, and zinc availability to ryegrass in a sewage sludge-amended field. Chemosphere 71:759–764.

Ashraf MY, Sadiq R, Hussain M, Ashraf M, Ahmad MSA (2011) Nickel contamination in growth medium disturbs the growth and metabolism in germinating seeds of sunflower (*Helianthus annuus* L.). Biol Trace Elem Res (In Press).

Assembly of Life Sciences (1975) U.S. Committee on Medical and Biologic Effects of Environmental Pollutants. Nickel. Published by National Academy of Sciences.

Atta-Aly MA (1999) Effect of nickel addition on the yield and quality of parsley leaves. Sci Hort 82:9–24.

Aubert H, Pinta M (1978) Trace Elements in Soils. Elsevier.

Aziz EE, Gad N, Badran NM (2007) Effect of cobalt and nickel on plant growth, yield and flavonoids content of *Hibiscus sabdariffa* L. Australian J Basic Appl Sci 1: 73–78.

Baccouch S, Chaoui A, El Ferjani E (1998a) Nickel toxicity: Effects on growth and metabolism of maize. J Plant Nutr 21:577–588.

Baccouch S, Chaoui A, El Ferjani E (1998b) Nickel-induced oxidative damage and antioxidant responses in *Zea mays* shoots. Plant Physiol Biochem 36:689–694.

Baccouch S, Chaoui A, El Ferjani E (2001) Nickel toxicity induces oxidative damage in *Zea mays* roots. J Plant Nutr 24:1085–1097.

Bai C, Reilly CC, Wood BW (2006a) Nickel deficiency disrupts metabolism of ureides, amino acids, and organic acids of young pecan foliage. Plant Physiol 140:433–443.

Bai C, Reilly CC, Wood BW (2007) Nickel deficiency affects nitrogenous forms and urease activity in spring xylem sap of pecan. J Amer Soc Hort Sci 132:302–309.

Bai C, Yi S, Reilly CC, Wood BW (2006b) Insights into the nutritional physiology of nickel. In: Proceedings of the International Journal of Horticultural Science. 27th International Horticultural Congress, August 13–19, 2006, Soul, Korea, p. 127–128.

Balaguer J, Almendo MB, Gomez I, Navarro-Pedreno J, Mataix J (1998) Tomato growth and yield affected by nickel presented in the nutrient solution, Acta Hort 458:269–272.

Bandurska H (2001) Does proline accumulated in leaves of water deficit stressed barley plants confine cell membrane injuries? II. Proline accumulation during hardening and its involvement

in reducing membrane injuries in leaves subjected to severe osmotic stress. Acta Physiol Plant 23:483–490.
Barcelo J, Poschenrieder C (1990) Plant water relations as affected by heavy metal stress: A review. J Plant Nut 13:1–37.
Barker AV (2006) Nickel. In: Barker AV, Pilbeam DJ (eds). Handbook of Plant Nutrition. CRC Press.
Bast E (1988) Nickel requirement for the formation of active urease in purple sulfur bacteria (Chromatiaceae). Arch Microbiol 150:6–10.
Bennett WF (1993) Nutrient Deficiencies and Toxicities in Crop Plants. St. Paul, MN. APS Press. PP: 202.
Benoit SL, Maier RJ (2003) Dependence of *Helicobacter pylori* urease activity on the nickel-sequestering ability of the UreE accessory protein. J Bacteriol 185:4787–95.
Benoit SL, Zbell AL, Maier RJ (2007) Nickel enzyme maturation in *Helicobacter hepaticus*: roles of accessory proteins in hydrogenase and urease activities. Microbiology 153:3748–3756.
Bewley JD (1997) Seed germination and dormancy. Plant Cell 9:1055–1066.
Bewley JD, Black M (1994) Seeds: Physiology of Development and Germination. 2nd Ed. Plenum Press, New York and London. pp: 1–31.
Bhatia NP, Orlic I, Siegele R, Ashwath N, Baker AJM, Walsh KB (2003) Elemental mapping using PIXE shows the main pathway of nickel movement is principally symplastic within the fruit of the hyperaccumulator *Stackhousia tryonii*. New Phytol 160:479–488.
Bhatia NP, Walsh KB, Baker AJM (2005) Detection and quantification of ligands involved in nickel detoxification in the herbaceous Ni hyperaccumulator *Stackhousia tryonii* Bailey. J Exp Bot 56:1343–1349.
Birch F (1964) Density and composition of the mantle and core. J Geophys Res 69(20): 4377–4388.
Bishnoi NR, Sheoran IS, Singh R (1993a) Effect of cadmium and nickel on mobilization of food reserves and activities of hydrolytic enzymes in germinating pigeon pea seeds. Biol Plant 35:583–589.
Bishnoi NR, Sheoran IS, Singh R (1993b) Influence of cadmium and nickel on photosynthesis and water relations in wheat leaves of different insertion level. Photosynthetica 28:473–479.
Blakely R, Zerner B (1984): Jack bean urease: the first nickel enzyme. J Mol Catal 23:263–292.
Boisvert S, Joly D, Leclerc S, Govindachary S, Harnois J, Carpentier R (2007) Inhibition of the oxygen-evolving complex of photosystem-II and depletion of extrinsic polypeptides by nickel. Biometals 20:879–889.
Bonam D, Ludden PW (1987) Purification and characterization of carbon monoxide dehydrogenase, a nickel, zinc, iron-sulfur protein, from *Rhodospirillum rubrum*. J Biol Chem 262:2980–2987.
Booker FL (2004) Influence of ozone on ribonuclease activity in wheat (*Triticum aestivum*) leaves. Physiol Plant, 120:249–255.
Boominathan R, Doran PM (2002) Ni-induced oxidative stress in roots of the Ni hyperaccumulator, *Alyssum bertolonii*. New Phytol 156:205–215.
Boyd RS, Wall MA, Jaffre T (2006) Nickel levels in arthropods associated with Ni hyperaccumulator plants from an ultramafic site in New Caledonia. Insect Sci 13(4):271–277.
Briggs DE (1992) Barley germination: Biochemical changes and hormonal control. In: Shewry PR (ed). Barley: Genetics, Biochemistry, Molecular Biology and Biotechnology, CAB International, Wallingford. Pp. 369–401.
Brooks RR, Shaw S, Marfil AA (1981) The chemical form and physiological function of nickel in some *Iberian Alyssum* species. Physiol Plant 51:167–170.
Brown PH (2006) Nickel. In: Barker AV, Pilbeam DJ (eds) Handbook of Plant Nutrition. CRC Press.
Brown PH, Welch RM, Cary EE (1987a) Nickel: A micronutrient essential for higher plants. Plant Physiol 85:801–803.
Brown PH, Welch RM, Cary EE, Checkai RT (1987b) Beneficial effects of nickel on plant growth. J Plant Nutr 10:2125–2135.
Brown PH, Welch RM, Madison JT (1990) Effect of nickel deficiency on soluble anion, amino acid, and nitrogen levels in barley. Plant Soil 125:19–27.
Burgdorf T, Lenz O, Buhrke T, van der Linden E, Jones AK, Albracht SPJ, Friedrich B (2005) [NiFe]-Hydrogenases of *Ralstonia eutropha* H16: Modular enzymes for oxygen-tolerant biological hydrogen oxidation. J Mol Microbiol Biotechnol 10:181–196 .

Burke DJ, Weis JS, Weis P (2000) Release of metals by the leaves of the salt marsh grasses *Spartina alterniflora* and *Phragmites australis*. Estuar Coasta Shelf Sci 51:153–159.

Cataldo DA, Garland TR, Wildung RE (1978) Nickel in plants: I. Uptake kinetics using intact soybean seedlings. Plant Physiol., 62: 563–565.

Cataldo DA, McFadden KM, Garland TR, Wildung RE (1988) Organic constituents and complexation of nickel (II), iron (III), cadmium (II) and plutonium (IV) in soybean xylem exudates. Plant Physiol 86:734–739.

Cempel M, Nikel G (2006) Nickel: A review of its sources and environmental toxicology. Polish J Environ Stud 15:375–382.

Chachalis D, Reddy KN (2000) Factors affecting *Campsis radicans* seed germination and seedling emergence. Weed Sci 48:212–216.

Chai SC, Ju T, Dang M, Goldsmith RB, Maroney MJ, Pochapsky TC (2008). Characterization of metal binding in the active sites of acireductone dioxygenase isoforms from *Klebsiella* ATCC 8724. Biochemistry 47:2428–38.

Chatterjee J, Chatterjee C (2000) Phytotoxicity of cobalt, chromium and copper in caulifower. Environ Poll 109:69–74.

Chawan DD (1995) Environment and Adaptive Biology of Plants. Scientific Publishers, Jodhpur, India.

Checkai RT, Norvell WA, Welch RM (1986) Investigation of nickel essentiality in higher plants using a recirculating resin-buffered hydroponic system. Agron Abst p 195.

Chen JC, Ting YY, Lin H, Lian TC (2009) Heavy metal. Concentrations in sea water from grass prawn hatcheries and the coast of Taiwan. J World Maricul Soc 16:316–332.

Clemens S (2001) Molecular mechanisms of plant metal tolerance and homeostasis. Planta 212:475–486.

Clugston SL, Barnard JFJ, Kinach R, Miedema D, Ruman R, Daub E, Honek JF (1998) Overproduction and characterization of a dimeric non-zinc glyoxylase I from *Escherichia coli* evidence for optimal activation by nickel ions. Biochemistry 37:8754–8763.

Clugston SL, Honek JF (2000) Identification of sequences encoding the detoxification metalloisomerase glyoxalase I in microbial genomes from several pathogenic organisms. J Mol Evol 50(5):491–5.

Cobbett CS (2000) Phytochelatins and their roles in heavy metal detoxification. Plant Physiol 123:825–833.

Collard JM, Corbisier P, Diels L, Dong Q, Jeanthon C, Mergeay M, Taghavi S, van der Lelie D, Wilmotte A, Wuertz S (1994) Plasmids for heavy metal resistance in *Alcaligenes eutrophus* CH_{34}: Mechanisms and applications, FEMS Microbiol Rev 14:405–414.

Colpas GJ, Hausinger RP (2000) In vivo and in vitro kinetics of metal transfer by the *Klebsiella aerogenes* urease nickel metallochaperone, UreE. J Biol Chem 275:10731–10737.

Costa M, Zhuang Z, Cosentino S, Klein CB, Salnikow K (1994) Molecular mechanisms of nickel carcinogenesis. Sci Total Environ 148:191–199.

Dahiya DJ, Singh JP, Kumar V (1993) Nitrogen uptake in wheat as influenced by the presence of nickel. Arid Land Res Manag 8:51–58.

Dalton DA, HJ Evans, FJ Hanus (1985) Stimulation by nickel of soil microbial urease activity and urease and hydrogenase activities in soybeans grown in a low-nickel soil. Plant Soil 88:245–258.

Darnault C, Volbeda A, Kim EJ, Legrand P, Vernède X, Lindahl PA, Fontecilla-Camps JC (2003) Ni-Zn-[Fe4-S4] and Ni-Ni-[Fe4-S4] clusters in closed and open alpha subunits of acetyl-CoA synthase/carbon monoxide dehydrogenase. Nat Struct Biol 10:271–279.

Dat J, Vandenabeele S, Vranova E, Van Montagu M, Inze D, Van Breusegem F (2000) Dual action of the active oxygen species during plant stress responses. Cell Mol Life Sci 57:779–795.

Demchenko NP, Kalimova IB, Demchenko KN (2005) Effect of nickel on growth, proliferation, and differentiation of root cells in *Triticum aestivum* seedlings. Russ J Plant Physiol 52:220–228.

DeMoll E, Grahame DA, Harnly JM, Tsai L, Stadtman TC (1987) Purification and properties of carbon monoxide dehydrogenase from *Methanococcus vannielii*. J Bacteriol 169: 3916–3920.

Dennis JK, Such TE (1993) Nickel and Chromium Plating, 3 rd ed. Cambridge, UK: Woodhead Publishing Ltd.

Diekert G, Ritter M (1983) Purification of the nickel protein carbon monoxide dehydrogenase of *Clostridium thermoaceticum*. FEBS Lett 151:41–44.

Diekert G, Thauer RK (1980) The effect of nickel on carbon monoxide dehydrogenase formation in *Clostridium thermoaceticum* and *Clostridium formicoaceticum*. FEMS Microbiol Lett 7:187–189.

Diekert G, Weber B, Thauer RK (1980) Nickel dependence of factor F430 content in *Methanobacterium thermoautotrophicum*. Arch Microbiol 127:273–278.

Dixon NE, Gazzola C, Blakely RL, Zemer R (1975) Jack bean urease (EC 3.5.1.5) A metalloenzyme. A simple biological role for nickel. J Am Chem Soc 97:4131–4133.

Dobrolyubskii OK, Slavvo AV (1957) Use of trace element nickel for the nutrition of grapes. Dokl Akad Nauk SSSR 112:347–359.

Doukov TI, Blasiak LC, Seravalli J, Ragsdale SW, Drennan CL (2008) Xenon In and at the End of the Tunnel of Bifunctional Carbon Monoxide Dehydrogenase/Acetyl-CoA Synthase. Biochem 47:3474–3483.

Drake HL, Hu S, Wood HG (1980) Purification of carbon monoxide dehydrogenase, a nickel enzyme from *Clostridium thermocaceticum*. J Biol Chem 255:7174–7180.

Duda-Chodak A, Baszczyk U (2008) The impact of nickel on human health. J Elementol 13(4): 685–696.

Eggen RI, Geerling AC, Jetten MS, de Vos WM (1991) Cloning, expression, and sequence analysis of the genes for carbon monoxide dehydrogenase of *Methanothrix soehngenii*. J Biol Chem 266:6883–6887.

Eitinger T (2004) *In vivo* production of active nickel superoxide dismutase from *Prochlorococcus marinus* MIT9313 is dependent on its cognate peptidase. J Bacteriol 186:7821–7825.

Eitinger T, Mandrand-Berthelot MA (2000) Nickel transport systems in microorganisms. Arch Microbiol 173:1–9.

El-Enany AE, Issa AA (2001) Proline alleviates heavy metal stress in *Scenedesmus armatus*. Folia Microbiol 46(3):227–230.

El-Shintinawy F, El-Ansary A (2000) Differential effect of Cd^{2+} and Ni^{2+} on amino acid metabolism in soybean seedlings. Biol Plant 43:79–84.

Ensink JHJ, Simmons RW, van der Hoek W (2007) Wastewater Use in Pakistan: The Cases of Haroonabad and Faisalabad. The International Development Research Centre, Canada. http://www.idrc.ca/fr/ev-68336-201-1-DO_TOPIC.html.

Ermler U, Grabarse W, Shima S, Goubeaud M, Thauer RK (1997) Crystal structure of methylcoenzyme M reductase: The key enzyme of biological methane formation. Science 278: 1457–1462.

Ermler U, Grabarse W, Shima S, Goubeaud M, Thauer RK (1998). Active sites of transition-metal enzymes with a focus on nickel. Curr Opin Struct Biol 8:749–758.

Eskew DL, Welch RM, Cary EE (1983) Nickel: An essential micronutrient for legumes and possibly all higher plants. Science 222:691–693.

Eskew DL, Welch RM, Norvell WA (1984) Nickel in higher plants: further evidence for an essential role. Plant Physiol 76:691–693.

Espen L, Pirovano L, Cocucci SM (1997) Effects of Ni^{2+} during the early phases of radish (*Raphanus sativas)* seed germination. Environ Exp Bot 38(2):187–197.

Ewais EA (1997) Effects of cadmium, nickel and lead on growth, chlorophyll content and proteins of weeds. Biol Plant 39:403–410.

Fismes J, Echevarria G, Leclerc-Cessac E, Morel JL (2005) Uptake and transport of radioactive nickel and cadmium into three vegetables after wet aerial contamination. J Environ Qual 34:1497–1507.

Foy CD, Chaney RL, White MC (1978) The physiology of metal toxicity in plants. Ann Rev Plant Physiol 29:511–566.

Freeman JL, Persans MW, Nieman K, Albrecht C, Peer W, Pickering IJ (2004) Increased glutathione biosynthesis plays a role in nickel tolerance in Thlaspi nickel hyperaccumulators. Plant Cell 16: 2176–2191.

Friedrich CG, Schneider K, Friedrich B (1982) Nickel in the catalytically active hydrogenase of *Alcaligenes eutrophus*. J Bacteriol 152:42–48.

Gabbrielli R, Pandolfini T, Espen L, Palandri MR (1999) Growth, peroxidase activity and cytological modifications in *Pisum sativum* seedlings exposed to Ni^{2+} toxicity. J Plant Physiol 155:639–645.

Gabbrielli R, Pandolfini T, Vergnano O, Palandri MR (1990) Comparison of two serpentine species with different nickel tolerance strategies. Plant Soil 122:271–277.

Gajewska E, Skłodowska M (2005) Antioxidative responses and proline level in leaves and roots of pea plants subjected to nickel stress. Acta Physiol Plant 27:329–339.

Gajewska E, Skłodowska M (2007) Effect of nickel on ROS content and antioxidative enzyme activities in wheat leaves. BioMetals 20:27–36.

Gajewska E, Skłodowska M (2008) Differential biochemical responses of wheat shoots and roots to nickel stress: Antioxidative reactions and proline accumulation. Plant Growth Regul 54:179–188.

Gajewska E, Skłodowska M, Słaba M, Mazur J (2006) Effect of nickel on antioxidative enzyme activities, proline and chlorophyll contents in wheat shoots. Biol Plant 50(4):653–659.

Galloway JN, Thornton JD, Norton SA, Volchok HL, McClean HL (1982) Trace metals in atmospheric deposition: A review and assessment. Atmo Environ 16:1677–1700.

Gaspar T, Penel C, Hagege D, Greppin H. 1991 Peroxidases in plant growth, differentiation, and development processes. In: Łobarzewski J, Greppin H, Penel C, Gaspar T (eds) Biochemical, Molecular and Physiological Aspects of Plant Peroxidases. Lublin, Poland: University M. Curie-Skłodowska, pp. 249–280.

Genrich I, Burd GI, Dixon DG, Glick BR (1998) A plant growth-promoting bacterium that decreases nickel toxicity in seedlings. Appl Environ Microbiol 64:3663–3668.

Gerendás J, Polacco JC, Freyermuth SK, Sattelmacher B (1999) Significance of nickel for plant growth and metabolism. J Plant Nutr Soil Sci 162:241–256.

Gerendás J, Sattelmacher B (1997a) Significance of N source (urea vs. NH_4NO_3) and Ni supply for growth, urease activity and nitrogen metabolism of zucchini (*Cucurbita pepo* convar. giromontiina).Plant Soil 196:217–222.

Gerendás J, Sattelmacher B (1997b): Significance of Ni supply for growth, urease activity and the contents of urea, amino acids and mineral nutrients of urea-grown plants. Plant Soil 190:153–162.

Gerendás J, Sattelmacher B (1999) Influence of Ni supply on growth and nitrogen metabolism of *Brassica napus* L. grown with NH_4NO_3 or urea as N source. Ann Bot 83: 65–71.

Gerendás J, Zhu Z, Sattelmacher B (1998) Influence of N and Ni supply on nitrogen metabolism and urease activity in rice (*Oryza sativa* L.). J Exp Bot 49:1545–1554.

Ghorbanli ML, Hoseiny RH, Shirdam RB, Khani SH (2006) The effect of nickel on seed germination proline metabolism, catalase and peroxides activity in *Brassica napus* L. J Environ Sci Technol 27:36–48.

Gomes-Filho E, Lima CRFM, Eneas-Filho J, Gondim LA, Prisco JT (1999) Purification and properties of a ribonuclease from cowpea cotyledons. Biol Plant 42:525–532.

Gonçalves SC, Portugal A, Gonçalves MT, Vieira R, Martins-Loução MA, Freitas H (2007) Genetic diversity and differential in vitro responses to Ni in *Cenococcum geophilum* isolates from serpentine soils in Portugal. Mycorrhiza, 17:677–686.

Gordon WR, Schwemmer SS, Hillman WS (1978) Nickel and the metabolism of urea by *Lemna paucicostata* Hegelm. Planta 140:265–268.

Graham RD, Welch RM, Walker CD (1985) A role of nickel in the resistance of plants to rust. Austr Agron Soc Proc 159.

Grahame DA, Stadtman TC (1987) Carbon monoxide dehydrogenase from *Methanosarcina barkeri*. Disaggregation, purification, and physicochemical properties of the enzyme. J Biol Chem 262:3706–3712.

Gray CW, Mclaren RG (2006) Soil factors affecting heavy metal solubility in some New Zealand soils. Water Air Soil Poll 175:3–14.

Hall JL (2002) Cellular mechanisms for heavy metal detoxification and tolerance, J Exp Bot 53:1–11.

Hammel KE, Cornwell KL, Diekert GB, Thauer RK (1984) Evidence for a nickel-containing carbon monoxide dehydrogenase in *Methanobrevibacter arboriphilicus*. J Bacteriol 157:975–978.

Harmens H, Hartog PR, ten Bookum WM, Verkleij JAC (1993) Increased zinc tolerance in *Silene vulgaris* (Moench) Garcke is not due to increased production of phytochelatins. Plant Physiol 103: 1305–1309.

Hasinur R, Shamima S Shah A, Shigenao KW (2005) Effects of nickel on growth and composition of metal micronutrients in barley plants grown in nutrient solution. J Plant Nutr 28:393–404.

Hausinger RP (1997) Metallocenter assembly in nickel-containing enzymes. J Biol Inorg Chem 2:279–286.

Haydon MJ, Cobbett CS. 2007. Transporters of ligands for essential metal ions in plants. New Phytol 174:499–506.

He MM, Clugston SL, Honek JF, Matthews BW (2000) Determination of the structure of *Escherichia coli* glyoxylase I suggests a structural basis for differential metal activation. Biochemistry 39:8719–8727.

He ZL, Yang XE, Stoffella PJ (2005) Trace elements in agroecosystems and impacts on the environment. J Med Biol 19:125–140.

Heale EL, Ormrod DP (1982) Effects of nickel and copper on *Acer rubrum*, *Cornus stolonifers*, *Lonicera tatarica*, and *Pinus resinosa.* Can J Bot 60:2674–2681.

Heath SM, Southworth D, D'Allura JA (1997) Localization of nickel in epidermal subsidiary cells of leaves of *Thlaspi montanum* var. Siskiyouense (Brassicaceae) using energy-dispersive X-ray microanalysis. Int J Plant Sci 158:184–188.

Hedfi A, Mahmoudi E, Boufahja F, Beyrem H, Aissa P (2007) Effects of increasing levels of nickel contamination on structure of offshore nematode communities in experimental microcosms. Bull Environ Contam Toxicol 79:345–349.

Hermle S, Vollenweider P, Günthardt-Goerg MS, Mcquattie CJ, Matyssek R (2007) Leaf responsiveness of *Populus tremula* and *Salix viminalis* to soil contaminated with heavy metals and acidic rainwater. Tree Physiol 27:1517–1531.

Hilgenkamp K (2005) Environmental Health: Ecological Perspectives. Jones and Bartlett Publishers.

Homer FA, Reeves RD, Brooks RR (1997) The possible involvement of amino acids in nickel chelation in some nickel-accumulating plants. Curr Top Phytochem 14:31–33.

Horak O (1985) The importance of nickel in Fabaceae II. Uptake and requirement of nickel by *Pisum sativum*. Phyton (Horn) 25:301–307.

Hostýnek JJ, Maibach HI (2002) Nickel and the Skin: Absorption, Immunology, Epidemiology, and Metallurgy. Informa Health Care Publishers.

Houlam C, Fares K (2001) Effect of salinity on seed germination and early seedling growth of sugar beet (*Beta vulgaris* L.). Seed Sci Technol 29(2):357–364.

Howlett NG, Avery SV (1997) Induction of lipid peroxidation during heavy metal stress in *Saccharomyces cerevisiae* and influence of plasma membrane fatty acid unsaturation. Appl Environ Microbiol 63(8):2971–2976.

Ishii M, Takishita S, Iwasaki T, Peerapornpisal Y, Yoshino J, Kodama T, Igarashi Y (2000) Purification and characterization of membrane-bound hydrogenase from *Hydrogenobacter thermop*hilus strain TK-6, an obligately autotrophic, thermophilic, hydrogen-oxidizing bacterium. Biosci Biotechnol Biochem 64:492–502 .

Jagetiya BL, Aery NI (1998) Effect of different nickel salts on germination in moong. Environ Develop 189–192.

Jarup L (2003) Hazards of heavy metal contamination. British Med. Bull 68:167–182.

Jaun B, Thauer RK (2007) Methyl-coenzyme M reductase and its nickel corphin coenzyme F430 in methanogenic archaea. In: Sigel A, Sigel H, Sigel RKO (eds) Nickel and its Surprising Impact in Nature. John Wiley and Sons, West Sussex, England. pp 323–356.

Jean L, Bordas F, Gautier-Moussard C, Vernay P, Hitmi A, Bollinger JC (2008) Effect of citric acid and EDTA on chromium and nickel uptake and translocation by *Datura innoxia.* Environ Pollut 153:555–563.

Jetten MS, Hagen WR, Pierik AJ, Stams AJ, Zehnder AJ (1991) Paramagnetic centers and acetyl-coenzyme A/CO exchange activity of carbon monoxide dehydrogenase from *Methanothrix soehngenii*. Eur J Biochem 195:385–391.

Jetten MSM, Stams AJM, Zehnder AJB (1989) Purification and characterization of an oxygen-stable carbon monoxide dehydrogenase of *Methanothrix soehngenii*. Eur J Biochem 181: 437–441.

Kanth BK, Oh TJ, Sohng JK (2010) Identification of two superoxide dismutases (FeSOD and NiSOD) from *Streptomyces peucetius* ATCC 27952. Biotechnol Bioprocess Engineer 15:785–792.

Karaca A (2004) Effect of organic wastes on the extractability of cadmium, copper, nickel, and zinc in soil. Geoderma 122(2–4):297–303.

Kastori R, Petrovi M, Petrovi N (1992) Effect of excess lead, cadmium, copper, and zinc on water relations in sunflower. J Plant Nutr 15:2427–2439.

Keeling SM, Stewart RB, Anderson CWN, Robinson BH (2003) Nickel and cobalt phytoextraction by the hyperaccumulator *Berkheya coddii* implications for polymetallic phytomining and phytoremediation. Int J Phytoremed 5:235–244.

Kerkeb L, Krämer U (2003) The role of free histidine in xylem loading of nickel in *Alyssum lesbiacum* and *Brassica juncea*. Plant Physiol 131:716–724.

Kevresan S, Petrovic N, Popovic M, Kandrac J (1998) Effect of heavy metals on nitrate and protein metabolism in sugar beet. Biol Plan 41:235–240.

Khalid BY, Tinsley J (1980) Some effects of nickel toxicity on ryegrass. Plant Soil 55:139–144.

Kim EJ, KimHP, Hah YC, Roe JH (1996) Differential expression of superoxide dismutases containing Ni and Fe/Zn in *Streptomyces Coelicolor*. Eur J Biochem 241: 178–185.

Kim S, Takahashi M, Higuchi K, Tsunoda K, Nakanishi H, Yoshimura E, Mori S, Nishizawa NK (2005) Increased nicotianamine biosynthesis confers enhanced tolerance of high levels of metals, in particular nickel, to plants. Plant Cell Physiol 46:1809–1818.

Kirchgessner M, Schnegg A (1979) Activity of proteases, leucine arylamidase and alpha-amylase in pancreatic tissue during nickel deficiency. Nutr Metab 23(1):62–4.

Kirkby EA, Römheld V (2004) Micronutrients in plant physiology: Functions, uptake and mobility. Proceedings No. 543, International Fertiliser Society, York, UK. 1–51.

Klucas RV, Hanus FJ, Russel SA, Evans HJ (1983) Nickel: A micronutrient element for hydrogen-dependent growth of *Rhizobium japonicum* and for expression of urease activity in soybean leaves. Proc Natl Acad Sci USA 80:2253–2257.

Kochian LV (1991) Mechanisms of micronutrient uptake and translocation in plants, In: Mortvedt JJ (eds), Micronutrients in Agriculture, Soil Science Society of America, Madison, WI, pp. 251–270.

Kopittke PM, Asher CJ, Blamey FPC, Menzies NW (2008) Tolerance of two perennial grasses to toxic levels of Ni^{2+}. Environ Chem 5:426–434.

Kopittke PM, Asher CJ, Menzies NW (2007) Toxic effects of Ni^{2+} on growth of cowpea (*Vigna unguiculata*). Plant Soil 292(2):283–289.

Körner LE, Møller LM, Jensén P (1987) Effects of Ca^{2+} and other divalent cations on uptake of Ni^{2+} by excised barley roots. Physiol Plant 71:49–54.

Kovacevi G, Kastori R, Merkulov LJ (1999) Dry matter and leaf structure in young wheat plants as affected by cadmium, lead, and nickel. Biol Plant 42:119–123.

Kowalczyk J, Borkowska-Burnecka J, Cieslak K (2003) Heavy metals accumulation in greenhouse tomatoes. Acta Hort 613:57–60.

Kramer PJ, Boyer JS (1995) Water Relations of Plants and Soils. Academic Press, Inc.

Krämer U, Cotter-Howells JD, Charnock JM, Baker AJM, Smith JAC (1996) Free histidine as a metal chelator in plants that accumulate nickel. Nature 379: 635–638.

Kravkina IM (2000) The chloroplast structure in leaf chlorenchyma cells of *Dianthus repens* (Caryophyllaceae) in response to high concentrations of soil nickel. Botanicheskii Zhurnal 85:83–85.

Krupa Z, Baszynski T (1995) Some aspects of heavy metals toxicity towards photosynthetic apparatus - direct and indirect effects on light and dark reactions. Acta Physiol Plant 17:177–190.

Krupa Z, Siedleka A, Maksymiec W, Baszynski T (1993) In vivo response of photosynthetic apparatus of *Phaseolus vulgaris* L. to nickel toxicity. J Plant Physiol 142:664–668.

Krzycki JA, Mortenson LE, Prince RC (1989) Paramagnetic centers of carbon monoxide dehydrogenase from aceticlastic *Methanosarcina barkeri*. J Biol Chem 264:7217–7221.

Krzycki JA, Zeikus JG (1984) Characterization and purification of carbon monoxide dehydrogenase from *Methanosarcina barkeri*. J Bacteriol 158:231–237.

Kukkola E, Rautio P, Huttunen S (2000) Stress indications in copper- and nickel-exposed Scots pine seedlings. Environ Exp Bot 43:197–210.

Küpper H, Kroneck PMH (2007) Nickel in the environment and its role in the metabolism of plants and cyanobacteria, In: Sigel A, Sigel H, Sigel RKO (eds), Metal Ions in Life Sciences, Wiley, Chichester. pp: 31–62.

Küpper H, Küpper F, Spiller M (1996) Environmental relevance of heavy metal-substituted chlorophylls using the example of water plants. J Exp Bot 47:259–266.

Küpper H, Lombi E,. Zhao FJ, Wieshammer G, McGrath SP (2001) Cellular compartmentation of nickel in the hyperaccumulators *Alyssum lesbiacum*, *Alyssum bertolonii* and *Thlaspi goesingense.* J Exp Bot 52:2291–2300.

Lavado RS (2006) Effects of sewage-sludge application on soils and sunflower yield: Quality and toxic element accumulation. J Plant Nutr 29:975–984.

Lee IS, Kim OK, Chang YY, Bae B, Kim HH, Baek KH (2002a) Heavy metal concentrations and enzyme activities in soil from a contaminated Korean shooting range. J Biosci Bioeng 94(5): 406–11.

Lee JW, Roe JH, Kang SO (2002b) Nickel-containing superoxide dismutase. Meth Enzymol 349: 90–101.

Leon V, Rabier J, Notonier R, Barthelemy R, Moreau X, Bouraima-Madjebi S, Viano J, Pineau R (2005) Effects of three nickel salts on germinating seeds of *Grevillea exul* var. rubiginosa, an endemic serpentine proteaceae. Ann Bot 95:609–618.

Lewinsky AA (2007) Hazardous Materials and Wastewater: Treatment, Removal and Analysis. Nova Publishers.

Li W, Khan MA, Yamaguchi S, Kamiya Y (2002) Effects of heavy metals on seed germination and early seedling growth of *Arabidopsis thaliana*. Plant Grow Regul 46:45–50.

Lin T, He X, Yang L, Shou H, Wu P (2005) Identification and characterization of a novel water-deficit-suppressed gene OsARD encoding an aci-reductone-dioxygenase-like protein in rice. Gene 360:27–34.

Lin YC, Kao CH (2006) Effect of excess nickel on starch mobilization in germinating rice grains. J Plant Nutr 29:1405–1412.

Lin YC, Kao CH (2007) Proline accumulation induced by excess nickel in detached rice leaves. Biol Plant 51:351–354.

Lindahl PA, Graham DE (2007) Acetyl-Coenzyme a synthases and nickel-containing carbon monoxide dehydrogenases. In: Sigel A, Sigel H, Sigel RKO (eds) Nickel and its Surprising Impact in Nature. John Wiley and Sons, West Sussex, England. pp 357–416.

Lindberg S, Greger M (2002) Plant genotypic differences under metal deficient and enriched conditions. In: Prasad MNV, Kazimierz S (eds), Physiology and Biochemistry of Metal Toxicity and Tolerance in Plants. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 357–393.

Liu D, Jiang W, Wang W, Zhai L (1995) Evaluation of metal ion toxicity on root tip cells by the *Allium* test. Israel J Plant Sci 43:125–133.

Liu WX (2008) Accumulation and translocation of toxic heavy metals in winter wheat (*Triticum aestivum* L.) growing in agricultural soil of Zhengzhou, China. Bull Environ Contam Toxicol 82:343–347.

Llamas A, Ullrich CI, Sanz A (2008) Ni^{2+} toxicity in rice: Effect on membrane functionality and plant water content, Plant Physiol Biochem 46: 905–910.

Long ER, MacDonald DD, Smith SL, Calder FD (1995) Incidence of adverse biological effects within ranges of chemical concentrations in marine and estuarine sediments. Environ Manag 19:81–97.

Lubitz W, Gastel MV, Gärtner W (2007) Nickel iron Hydrogenases. In: Sigel A, Sigel H, Sigel RKO (eds) Nickel and its Surprising Impact in Nature. John Wiley and Sons, West Sussex, England. pp 279–322.

Mackay EM, Pateman JA (1980) Nickel requirement of a urease-deficient mutant in *Aspergillus nidulans*. J Gen Microbiol 116:249–251.
MacNicol RD, Beckett PHT (1985) Critical tissue concentrations of potentially toxic elements. Plant Soil 85:107–129.
Maheshwari R, Dubey R (2008) Inhibition of ribonuclease and protease activities in germinating rice seeds exposed to nickel. Acta Physiol Plant 30(6):863–872.
Maheshwari R, Dubey RS (2007) Nickel toxicity inhibits ribonuclease and protease activities in rice seedlings: Protective effects of proline. Plant Grow Regul 51:231–243.
Maheshwari R, Dubey RS (2009) Nickel-induced oxidative stress and the role of antioxidant defence in rice seedlings. Plant Grow Regul 59:37–49.
Mahlert F, Bauer C, Jaun B, Thauer RK, Duin EC (2002) The nickel enzyme methyl-coenzyme M reductase from methanogenic archaea: In vitro induction of the nickel-based MCR-ox EPR signals from MCR-red2. J Biol Inorg Chem7:500–513.
Maier RJ, Benoit SL, Seshadri S (2007) Nickel-binding and accessory proteins facilitating Ni-enzyme maturation in *Helicobacter pylori*. Biometals 20:655–664.
Maier T, Jacobi A, Sauter M, Bock A (1993) The product of the hypB gene, which is required for nickel incorporation into hydrogenases, is a novel guanine nucleotide protein. J Bacteriol 175:630–635.
Malkin R, Niyogi K (2000) Photosynthesis. In Buchanan BB, Gruissem W, Jones RL (eds), Biochemistry and Molecular Biology of Plants. American Society of Plant Physiologists, Rockville, MD, Pp 568–628.
Marschner H (1995) Mineral Nutrition of Higher Plants, 2nd Ed. Academic Press, London. pp: 889.
Matraszek R, Szymanska M, Wroblewska M (2002) Effect of nickel on yielding and mineral composition of the selected vegetables. Acta Scientiarum Polonorum - Technica Agraria 1: 3–22.
Matysik J, Alia, Bhalu B, Mohanty P (2002) Molecular mechanisms of quenching of reactive oxygen species by proline under stress in plants. Curr Sci 82:525–532.
Mayer AM, Poljakoff-Mayber A (1975) The Germination of Seeds. New York: Pergamon Press.
McIlveen WD, Negusanti JJ (1994) Nickel in the terrestrial environment. Sci Total Environ 148:109–138.
Mehta N, Olson JW, Maier RJ (2003) Characterization of *Helicobacter pylori* nickel metabolism accessory proteins needed for maturation of both urease and hydrogenase. J. Bacteriol. 185: 726–734.
Mehta SK, Gaur JP (1999) Heavy-metal-induced proline accumulation and its role in ameliorating metal toxicity in *Chlorella vulgaris*. New Phytol 143:253–259.
Menon MSH, Abbaspour KC, Gunthardt-Goerg MS, Oswald SE, Schulin R (2005) Water regime of metal-contaminated soil under juvenile forest vegetation. Plant Soil 271:227–241.
Mishra D, Kar M (1974) Nickel in plant growth and metabolism. Bot Rev 40:395–452.
Misra SG, Pande P (1974) Effect of organic matter on availability of nickel. Plant Soil 40:679–684.
Mizuno N, Nosaka S, Mizuno T, Horie K, Obata H (2003) Distribution of Ni and Zn in the leaves of *Thlaspi japonicum* growing on ultramafic soil. Soil Sci Plant Nutr 49:93–97.
Mohanty N, Vaas I, Demeter S (1989) Impairment of photosystem II: Activity at the level of secondary quinone electron acceptor in chloroplasts treated with cobalt, nickel and zinc ions. Physiol Plant 76:386–390.
Molas J (1997) Changes in morphological and anatomical structure of cabbage (*Brassica oleracea* L.) outer leaves and in ultrastructure of their chloroplasts caused by an *in vitro* excess of nickel. Photosynthetica 34:513–522.
Molas J (2002) Changes of chloroplast ultrastructure and total chlorophyll concentration in cabbage leaves caused by excess of organic Ni (II) complexes. Environ Exp Bot 47:115–126.
Montarges-Pelletier E, Chardot V, Echevarria G, Michot LJ, Bauer A, Morel JL (2008) Identification of nickel chelators in three hyperaccumulating plants: An X-ray spectroscopic study. Phytochemistry 69:1695–1709.

Moraes MF, Reis AR, Moraes LAC, Lavres-Junior J, Vivian R, Cabral CP, Malavolta E (2009) Effects of molybdenum, nickel, and nitrogen sources on the mineral nutrition and growth of rice plant. Commun Soil Sci Plant Analy 40: 3238–3251.

Morton TA, Runquist JA, Ragsdale SW, Shanmugasundaram T, Wood HG and Ljungdahl LG (1991) The primary structure of the subunits of carbon monoxide dehydrogenase/acetyl-CoA synthase from *Clostridium thermoaceticum*. J Biol Chem 266:23824–23828 .

Mott JJ (1974) Factors affecting seed germination in three annual species from an arid region of western australia. J Ecol 62:699–709.

Moya JL (1995) Heavy metal-hormone interactions in rice plants: Effects on growth, net photosynthesis, and carbohydrate distribution. J Plant Grow Regul 14:61–67.

Mulrooney SB, Hausinger RP (2003) Nickel uptake and utilization by microorganisms, FEMS Microbiol Rev 27:239–261.

Munzuroglu O, Geckil H (2002) Effects of metals on seed germination, root elongation, and coleoptile and hypocotyl Growth in *Triticum aestivum* and *Cucumis sativus.* Arch Environ Contam Toxicol 43:203–213.

Murch SJ, Haq K, Rupasinghe HPV, Saxena PK (2003) Nickel contamination affects growth and secondary metabolite composition of St. John's wort (*Hypericum perforatum* L.). Environ Exp Bot 49:251–257.

Muyssen BTA, Brix KV, DeForest DK, Janssen CR (2004) Nickel essentiality and homeostasis in aquatic organisms. Environ Rev 12:113–131.

Nakazawa R, Kameda Y, Ito T, Ogita Y, Michihata R, Takenaga H (2004) Selection and characterization of nickel-tolerant tobacco cells. Biol Plant 48: 497–502.

National Pollutant Inventory (1999) Technical Advisory Panel Report. Department of the Environment, Water, Heritage and the Arts, GPO Box 787, Parkes ACT 2600, Australia .

Nedhi A, Singh LJ, Singh SI (1990) Effect of cadmium and nickel on germination, early seedling growth and photosynthesis of wheat and pigeon pea. Ind J Trop Agri 8:141–147.

Neumann PM, Chamel A (1986) Comparative phloem mobility of nickel in nonsenescent plants. Plant Physiol 81:689–691.

Nielsen, F.H. 1993. Ultratrace Minerals. Chap. 15. pp. 269–286. In: Shils ME, Olson JA, Shike M, Ross AC (eds) Modern Nutrition in Health and Disease, 8th Ed. Lea & Febiger, Philadelphia.

Nieminen T, Helmisaari HS (1996) Nutrient retranslocation in the foliage of *Pinus sylvestris* L. growing along a heavy metal pollution gradient. Tree Physiol 16:825–831.

Noctor G, Foyer CH (1998) Ascorbate and glutathione: Keeping active oxygen under control. Annu Rev Plant Physiol Plant Mol Biol 49:249–279.

Nriagu JO (1989) A global assessment of natural sources of atmospheric trace metals. Nature 338:47–49.

Nriagu JO, Pacyna JF (1988) Quantitative assessment of worldwide contamination of air, water, and soils by trace metals. Nature 333:134–139.

Ogata H, Mizoguchi Y, Mizuno N, Miki K, Adachi SI, Yasuoka N, Yagi T, Yamauchi O, Hirota S, Higuchi Y (2002) Structural studies of the carbon monoxide complex of [NiFe] hydrogenase from *Desulfovibrio vulgaris* Miyazaki F: Suggestion for the initial activation site for dihydrogen. J Am Chem Soc 124:11628–11635.

Oller AR, Costa M, Oberdörster G (1997) Carcinogenicity assessment of selected nickel compounds. Toxicol Appl Pharmacol 143:152–166.

Olson JW, Fu C, Maier RJ (1997) The hypB protein from *Bradyrhizobium japonicum* can store nickel and is required for the nickel-dependent transcriptional regulation of hydrogenase. Mol Microbiol 24:119–128.

Olson JW, Mehta NS, Maier RJ (2001) Requirement of nickel metabolism proteins HypA and HypB for full activity of both hydrogenase and urease in *Helicobacter pylori*. Mol Microbiol 39: 176–182.

Page V, Feller U (2005) Selective transport of zinc, manganese, nickel, cobalt and cadmium in the root system and transfer to the leaves in young wheat plants. Ann Bot 96:425–434.

Page V, Weisskopf L, Feller U (2006) Heavy metals in white Lupin: Uptake, root-to-shoot transfer and redistribution within the plant. New Phytol 171:329–341.
Palacios G, Gomez I, Carbonell-Barrachina A, Pedreno JN, Mataix J (1998) Effect of nickel concentration on tomato plant nutrition and dry matter yield. J. Plant Nutr 21:2179–2191.
Paleg LG, Stewart GR, Bradbeer JW (1984) Proline and glycine betaine influence protein solvation. Plant Physiol 75:974–78.
Palma JM, Sandalio LM, Corpas FJ, Romero-Puertas MC, McCarthy I, del Rio LA (2002) Plant protease, protein degradation, and oxidative stress: Role of peroxisomes. Plant Physiol Biochem 40:521–530.
Pandey N, Sharma CP (2002) Effect of heavy metals Co^{2+}, Ni^{2+} and Cd^{2+} on growth and metabolism of cabbage. Plant Sci 163:753–758.
Pandolfini T, Gabbrielli R, Comparini C (1992) Nickel toxicity and peroxidase activity in seedlings of *Triticum aestivum* L. Plant Cell Environ 15:719–725.
Papazogloua EG, Serelisb KG, Bouranisc DL (2007) Impact of high cadmium and nickel soil concentration on selected physiological parameters of *Arundo donax* L. European J Soil Biol 43:207–215.
Patterson W, Olson JJ (1983) Effects of heavy metals on radicle growth of selected woody species germinated on filter paper, mineral and organic soil substrates. Canadian J Forest Res 13:233–238.
Pena LB, Tomaro ML, Gallego SM (2006) Effect of different metals on protease activity in sunflower cotyledons, Electron J Biotechnol.9:258–262.
Peralta-Videa JR, Gardea-Torresdey JL, Gomez E, Tiemann KJ, Parsons JG, Carrillo G (2002) Effect of mixed cadmium, copper, nickel and zinc at different pHs upon alfalfa growth and heavy metal uptake. Environ Pollut 119:291–301.
Peralta-Videa JR, Gardea-Torresdey JL, Tiemann KJ, Gomez E, Arteaga S, Rascon E, Parsons JG (2001) Uptake and effects of five heavy metals on seed germination and plant growth in alfalfa (*Medicago sativa* L.). Bull Environ Contam Toxicol 66:727–734.
Pérezurria E, Legaza ME, Vicente C (1986) The function of nickel on the urease activity of the lichen *Evernia prunastri*. Plant Sci 43:37–43.
Pianelli K, Mari S, Marques L, Lebrun M, Czernic P (2005) Nicotianamine over-accumulation confers resistance to nickel in *Arabidopsis thaliana.* Transgen Res 14:739–748.
Piccini DF, Malavolta E (1992) Effect of nickel on two common bean cultivars. J Plant Nutr 15:2343–2350.
Pochapsky TC, Ju T, Dang M, Beaulieu R, Pagani GM, Ouyang B (2007) Nickel in acireductone dioxygenase in nickel and its surprising impact in nature. In: Sigel A, Sigel H, Sigel RKO (eds) Nickel and its Surprising Impact in Nature. John Wiley and Sons, West Sussex, England. pp 473–500.
Pochapsky TC, Pochapsky SS, Ju T, Mo H, Al-Mjeni F, Maroney MJ (2002) Modeling and experiment yields the structure of acireductone dioxygenase from *Klebsiella pneumoniae*. Nat Struct Biol 9:966–972.
Polacco JC (1976) Nitrogen metabolism in soybean tissue culture: I. Assimilation of urea. Plant Physiol 58(3): 350–357.
Polacco JC (1977) Nitrogen metabolism in soybean tissue culture: II. Urea utilization and urease synthesis require Ni^{2+}. Plant Physiol 59(5): 827–830.
Polacco JC, Havir EA (1979) Comparisons of soybean urease isolated from seed and tissue culture. J Biol Chem 254(5):1707–1715.
Polacco JC, Sparks RB (1982) Patterns of urease synthesis in developing soybeans. Plant Physiol 70(1):189–194.
Polacco JC, Thomas AL, Bledsoe PJ (1982) A soybean seed urease-null produces urease in cell culture. Plant Physiol 69(5):1233–1240.
Polacco JC, Winkler RG (1984) Soybean leaf urease: A seed enzyme?. Plant Physiol 74(4): 800–803.
Ragsdale S (2002) Nickel containing CO dehydrogenases and hydrogenases. Subcell Biochem 35:487–518.
Ragsdale SW (1998) Nickel biochemistry, Curr Opin Chem Biol 2:208–215.

Ragsdale SW (2003) Biochemistry of methyl-CoM reductase and coenzyme F430. In: Kadish KM, Smith KM, Guilard R (eds) The Porphyrin Handbook. Academic Press, New York. pp 205–228.

Ragsdale SW (2009) Nickel-based enzyme systems. J Biol Chem 284:18571–18575.

Ragsdale SW, Clark JE, Ljungdahl LG, Lundie LL, Drake HL (1983a) Properties of purified carbon monoxide dehydrogenase from *Clostridium thermoaceticum*, a nickel, iron-sulfur protein. J Biol Chem 258:2364–2369.

Ragsdale SW, Ljungdahl LG, DerVartanian DV (1983b) Isolation of carbon monoxide dehydrogenase from *Acetobacterium woodii* and comparison of its properties with those of the *Clostridium thermoaceticum* enzyme. J Bacteriol 155:1224–1237.

Ragsdale SW, Wood HG, Antholine WE (1985) Evidence that an iron-nickel-carbon complex is formed by reaction of CO with the CO dehydrogenase from *Clostridium thermoaceticum*. PNAS: 82:6811–6814.

Rahman H, Sabreen S, Alam S, Kawai S (2005) Effects of nickel on growth and composition of metal micronutrients in barley plants grown in nutrient solution. J Plant Nutr 28:393–404.

Randhawa VK, Fengzhen Z, Xiaolei J, Nalewajko C, Kushner DJ (2001) Role of oxidative stress and thiol antioxidant enzymes in nickel toxicity and resistance in strains of the green alga *Scenedesmus acutus* f. alternans, Can J Microbiol 47: 987–993.

Ranieri A, Castagna A, Pacini J, Baldan B, Sodi AM, Soldatini GF (2003) Early production and scavenging of hydrogen peroxide in the apoplast of sunflowers plants exposed to ozone. J Exp Bot 54: 2529–2540.

Rao KVM, Sresty TVS (2000) Antioxidative parameters in the seedlings of pigeon pea (*Cajnus cajan* (L.) Millspaugh) in response to Zn and Ni stresses. Plant Sci 157:113–128.

Rauser WE, Dumbroff EB (1981) Effects of excess cobalt, nickel and zinc on the water relations of *Phaseolus vulgaris*. Environ Exp Bot 21:249–255.

Rauta C, Ionescu A, Lacatusu R (1995) Studies on the translocation of nickel in maize and soyabean plants grown in polluted soils. Cercetari Agronomice in Moldova 28:15–22.

Rautaray SK, Ghosh BC, MittraBN (2003) Effect of fly ash, organic wastes and chemical fertilizers on yield, nutrient uptake, heavy metal content and residual fertility in a rice-mustard cropping sequence under acid lateritic soils. Bioresour Technol 90(3):275–283.

Reddy KJ (2006) Physiology and Molecular Biology of Stress Tolerance in Plants. In: Nutrient Stress. Springer, The Netherlands. Pp: 187–217. .

Rees TAV, Bekheet IA (1982) The role of nickel in urea assimilation by algae. Planta 156:385–387.

Reeves RD, Baker AJM, Bgrhidi A, Berazaín R (1996) Nickel-accumulating plants from the ancient serpentine soils of Cuba. New Phytol 133:217–224.

Reilly CC, Wood BW, Bai C (2006) Beneficial effects of nickel in pecan production. Pecan Grower 17:26–27 .

Riesen O, Feller U (2005) Redistribution of nickel, cobalt, manganese, zinc and cadmium via the phloem in young and in maturing wheat. J Plant Nutr 28:421–430.

Roach WA, Barclay C (1946) Nickel and multiple trace-element deficiencies in agricultural crops. Nature 157:696.

Robertson AI, Meakin MER (1980) The effect of nickel on cell division and growth of *Brachystegia spiciformis* seedlings. J Bot Zimbabwe 12:115–125.

Rodrigues R, Valente FM, Pereira IA, Oliveira S, Rodrigues-Pousada C (2003) A novel membrane-bound Ech [NiFe] hydrogenase in *Desulfovibrio gigas*. Biochem Biophys Res Commun 306:366–375.

Ros R, Cooke DT, Burden RS, James CS (1990) Effects of the herbicide MCPA, and the heavy metals, cadmium and nickel on the lipid composition, Mg^{2+}- ATPase activity and fluidity of plasma membranes from rice, *Oryza sativa* (cv. Bahia) shoots. J Exp Bot 41:457–462.

Rospert S, Breitung J, Ma K, Schwörer B, Zirngibl C, Thauer RK, Linder D, Huber R, Stetter KO (1991) Methyl-coenzyme M reductase and other enzymes involved in methanogenesis from CO_2 and H_2 in the extreme thermophile *Methanopyrus kandleri*. Arch Microbiol 156: 49–55.

Rubio MI, Escrig I, Martinez-Cortina C, Lopez-Benet FJ, Sanz A (1994) Cadmium and nickel accumulation in rice plants. Effects on mineral nutrition and possible interactions of abscisic and gibberellic acids. Plant Grow Reg 14:151–157.

Sabir M, Ghafoor A, Saifullah, Rehman MZU, Ahmad HR, Aziz T (2011) Growth and metal ionic composition of *Zea mays* as affected by nickel supplementation in the nutrient solution. Int J Agric Biol 13:186–190.

Sakamoto T, Bryant DA (2001) Requirement of nickel as an essential micronutrient for the utilization of urea in the marine cyanobacterium *Synechococcus* sp. PCC 7002. Microb Environ 16:177–184.

Sauter M, Lorbiecke R, Ouyang B, Pochapsky TC, Rzewuski G (2005) The immediate-early ethylene response gene OsARD1 encodes an acireductone dioxygenase involved in recycling of the ethylene precursor S-adenosylmethionine. Plant J 44:718–29.

Schmidt A, Schmidt A, Haferburg G, Kothe E (2007) Superoxide dismutases of heavy metal resistant streptomycetes. J Basic Microbiol 47:56–62.

Schor-Fumbarov T, Goldsbrough PB, Adam Z, Tel-Or E (2005) Characterization and expression of a metallothionein gene in the aquatic fern *Azolla filiculoides* under heavy metal stress. Planta 223:69–76.

Seravalli J, Xiao Y, Gu W, Cramer SP, Antholine WE, Krymov V, Gerfen GJ, Ragsdale SW (2004) Evidence that Ni-Ni Acetyl-CoA synthase is active and that the Cu-Ni enzyme is not. Biochemistry 43:3944–3955.

Seregin IV, Kozhevnikova AD (2005) Distribution of cadmium, lead, nickel, and strontium in imbibing maize caryopses. Russian J Plant Physiol 52:565–569.

Seregin IV, Kozhevnikova AD (2006) Physiological role of nickel and its toxic effects on higher plants. Russian J Plant Physiol 53:257–277.

Seregin IV, Kozhevnikova AD, Kazyumina EM, Ivanov VB (2003) Nickel toxicity and distribution in maize roots, Fiziol Rast 50:793–800.

Setia RC, Bala R (1994) Anatomical changes in root and stem of wheat (*Triticum aestivwn* L.) in response to different heavy metals. Phytomorph 44:95–104.

Sharma CP, Sharma PN (1987) Mineral nutrient deficiencies affect plant water relations. J Plant Nutr 10:9–16.

Sharma P, Dubey RS (2007) Involvement of oxidative stress and role of antioxidative defense system in growing rice seedlings exposed to toxic concentrations of aluminum. Plant Cell Rep 26:2027–2038.

Sharma PD (2005) Environmental Biology and Toxicology. Rastogi Publications.

Sharma SS, Dietz KJ (2006) The significance of amino acids and amino acid-derived molecules in plant responses and adaptation to heavy metal stress. J Exp Bot 57:711–726.

Sheoran IS, Singal HR, Singh R (1990) Effect of cadmium and nickel on photosynthesis and the enzymes of the photosynthetic carbon reduction cycle in pigeonpea (*Cajanus cajan* L.). Photosynth Res 23:345–351.

Shi GR, Cai QS (2008) Photosynthetic and anatomic responses of peanut leaves to cadmium stress. Photosynthetica 46(4): 627–630.

Shimada N, Ando T, Tomiyama M, Kaku H (1980) Role of nickel in plant nutrition. Effects of nickel on the growth of tomato and soybean. Japanese J Soil Sci Plant Nutr 51:493–496.

Shukla R, Gopal R (2009) Excess nickel alters growth, metabolism, and translocation of certain nutrients in potato. J Plant Nutr 32:1005–1014.

Sigel A, Sigel H, Sigel RKO (2005) Biogeochemistry, Availability, and Transport of Metals in the Environment. Informa Health Care Publishers.

Sigel H, Sigel A (1994) Handbook on Metals in Clinical and Analytical Chemistry. CRC Press.

Singh DP, Khare P, Singh PS (1989) Effect of Ni^{2+}, Hg^{2+} and Cu^{2+} on growth, oxygen evolution and photosynthetic electron transport in *Cylindrospermum* IU 942, J Plant Physiol 134:406–412.

Singh P, Nayyar K (2001) Influence of lime on nickel availability to plants and its toxic level in cowpea. J Res Punjab Agri Univ 38:10–13.

Singh S, Saxena R, Pandey K, Bhatt K, Sinha S (2004) Response of antioxidants in sunflower (*Helianthus annuus* L.) grown on different amendments of tannery sludge: Its metal accumulation potential. Chemosphere 57:1663–1673.

Singh SH (1984) Effects of nickel on germination and growth, total nitrogen and phosphate levels of *Cicer arietinum* seedlings. Trop Ecol 25:90–94.
Smirnoff N, Stewart GR (1987) Nitrogen assimilation and zinc toxicity to zinc-tolerant and non-tolerant clones of Deschampsia cespitosa (L.) Beauv. New Phytol 107:671–680.
Smith RM, Martell AE (1989) Stability Constants, Vol. 6: Second Supplement, Plenum Press, New York.
Smith SL, MacDonald DD, Keenleyside KA, Ingersoll CG, Field LJ (1996) A preliminary evaluation of sediment quality assessment values for freshwater ecosystems. J Great Lakes Res 22:624–638.
Smith SR (1994) Effect of soil pH on availability to crops of metals in sewage sludge-treated soils I: Nickel, copper, and zinc uptake and toxicity to ryegrass. Environ Poll 85:321–327.
Soom C, Rumjanek N, Vanderleyden J, Neves MCP (1993) Hydrogenase in *Bradyrhizobium japonicum*: Genetics, regulation and effect on plant growth. World J Microbiol Biotechnol 9:615–624.
Spears JW, Smith CJ, Hatfield EE (1977) Rumen bacterial urease requirement for nickel. J Dairy Sci 60:1073–1076.
Sresty TVS, Rao KVM (1999) Ultrastructural alterations in response to zinc and nickel stress in the root cells of pigeon pea. Environ Exp Bot 41:3–13.
Steffens JC (1990) The heavy metal-binding peptides of plants. Annu Rev Plant Physiol Plant Mol Biol 41:553–575.
Stixrude L, Waserman E, Cohen R (1997) Composition and temperature of Earth's inner core. J Geophys Res 102 (B11): 24729–24740.
Sukdeo N, Daub E, Honek JF (2007) Biochemistry of the nickel-dependent glyoxalase I enzymes. In: Sigel A, Sigel H, Sigel RKO (eds) Nickel and its Surprising Impact in Nature, John Wiley and Sons, West Sussex, England. pp 445–472.
Sukkariyah BF, Evanylo G, Zelazny L, Chaney RL (2005) Cadmium, copper, nickel, and zinc availability in a biosolids-amended piedmont soil years after application. J Environ Qual 34:2255–2262.
Sullivan JB, GR Krieger (2001) Clinical Environmental Health and Toxic Exposures. Lippincott Williams and Wilkins Publishers.
Sun E, Wu F (1998) Along-vein necrosis as indicator symptom on water spinach caused by nickel in water culture. Bot Acad Sin 39:255–259.
Sunderman F (1992) Toxicokinetics of nickel in humans. In: Nieboer E, Nriagu JO (eds). Nickel and Human Health: Current Perspectives. Vol 25, John Wiley & Sons Inc., New York, Pp: 69–76.
Svetlitchnyi V, Dobbek H, Meyer-Klaucke W, Meins T, Thiele B, Römer P, Huber R, Meyer O (2004) A functional Ni-Ni-[4Fe-4 S] cluster in the monomeric acetyl-CoA synthase from *Carboxydothermus hydrogenoformans*. PNAS : 101:446–451.
Syros T, Yupsanis T, Petkou D, Economou AS (2005) Protein, leucine aminopeptidase, esterase, acid phosphatase and photosynthetic responses of oleander (*Nerium oleander* L.) during cold acclimation and freezing treatments. J Plant Physiol 162:886–894.
Szalontai B, Horvath LI, Debreczeny M, Droppa M, Horvath G (1999) Molecular rearrangements of thylakoids after heavy metal poisoning, as seen by fourier transform infrared (FTIR) and electron spin resonance (ESR) spectroscopy, Photosynth Res 61:241–252.
Tabatabaei SJ (2009) Supplements of nickel affect yield, quality, and nitrogen metabolism when urea or nitrate is the sole nitrogen source for cucumber. J Plant Nutr 32:713–724.
Taiz L, Zeiger E (2006) Plant Physiology (4nd ed.). Sinauer Associates, Inc. Sunderland, Massachusetts, pp. 103–122.
Tan XW, Ikeda H, Oda M (2000) Effects of nickel concentration in the nutrient solution on the nitrogen assimilation and growth on tomato seedlings in hydroponic culture supplied with urea or nitrate as the sole nitrogen solution. Sci Hort 84:265–273.
Terlesky KC, Nelson MJ, Ferry JG (1986) Isolation of an enzyme complex with carbon monoxide dehydrogenase activity containing corrinoid and nickel from acetate-grown *Methanosarcina thermophila*. J Bacteriol 168:1053–1058.
Tersteegen A, Hedderich R (1999) *Methanobacterium thermoautotrophicum* encodes two multi-subunit membrane-bound [NiFe] hydrogenases. Transcription of the operons and sequence analysis of the deduced proteins. Eur J Biochem 264:930–43.

Tripathy BC, Bhatia B, Mohanty P (1981) Inactivation of chloroplast photosynthetic electron transport activity by Ni^{2+}. Biochim Biophys Acta 638:217–224.
Vacchina V, Mari S, Czernic P, Marques L, Pianelli K, Schaumloffel D, Lebrun M, Lobinski R (2003) Speciation of nickel in a hyperaccumulating plant by high performance liquid chromotgraphy-inductively coupled plasma mass spectrometry and electrospray MS/MS assisted by cloning using yeast complementation. Anal Chem 75:2740–2745.
Valko M, Morris H, Cronin MTD (2005) Metals: toxicity and oxidative stress. Curr Med Chem 12:1161–1208.
Van Assche F, Clijsters H (1990) Effects of metals on enzyme activity in plants. Plant Cell Environ 13:195–206.
Van den Ende W, Michiels A, Le Roy K, Van Laere A (2002) Cloning of a vacuolar invertase from *Belgian endive* leaves (*Cichorium intybus*). Physiol Plant 115:504–512.
van der Hoek W, Hassan MUl, Ensink JHJ, Feenstra S, Raschid-Sally L, Munir S, Aslam R, Ali N, Hussain R, Matsuno Y (2002) Urban Wastewater: A Valuable Resource for Agriculture A Case Study from Haroonabad, Pakistan. IWMI Research Report no. 63, International Water Management Institute, Colombo, Sri Lanka, pp 14.
Veer B (1989) Effect of nickel and zinc on seedling growth and hydrolytic enzymes in *Phaseolus aureus* cv. R-851. Geobios 16:245–248.
Veeranjaneyulu K, Das VSR (1982) Intrachloroplast localization of ^{65}Zn and ^{63}Ni in a Zn-tolerant plant, *Ocimum basilicum* Benth., J Exp Bot 33:1161–1165.
Verma S, Dubey RS (2003) Lead toxicity induces lipid peroxidation and alters the activities of antioxidant enzymes in growing rice plants. Plant Sci 164: 645–655.
Vernay P, Gauthier-Moussard C, Hitmi A (2007) Interaction of bioaccumulation of heavy metal chromium with water relation, mineral nutrition and photosynthesis in developed leaves of *Lolium perenne* L. Chemosphere 68: 1563–1575.
Vogel-Mikus K, Drobne D, Regvar M (2005) Zn, Cd and Pb accumulation and arbuscular mycorrhizal colonization of pennycress *Thlaspi praecox* Wulf. (Brassicaceae) from the vicinity of a lead mine and smelter in Slovenia. Environ Pollut 133:233–242.
Voss RD (1993) Corn. In: Nutrient Deficiencies and Toxicities in Crop Plants. St. Paul, MN. APS Press. P. 202.
Walker CD, Graham RD, Madison JT, Cary EE, Welch RM (1985) Effects of nickel deficiency on some nitrogen metabolites in cowpeas (*Vigna unguiculata* L. Walp). Plant Physiol 79:474–479.
Wang HH, Kang J, Zeng FH, Jiang MY (2001) Effect of nickel at high concentrations on growth and activities of enzymes of rice seedlings. Acta Agron Sin 27:953–957.
Wang ST, He XJ, An RD (2010) Responses of growth and antioxidant metabolism to nickel toxicity in *Luffa cylindrica* seedlings. J Animal Plant Sci 7: 810–821.
Wang Z, Zhang Y, Huang Z, Huang L (2008) Antioxidative response of metal-accumulator and non-accumulator plants under cadmium stress. Plant Soil 310:137–149.
Watt RK, Ludden PW (1998) The identification, purification, and characterization of Cool. A nickel-binding protein that is co-regulated with the Ni-containing CO dehydrogenase from *Rhodospirillum rubrum.* J Biol Chem 273:10019–10025.
Welch RM (1981) The biological significance of nickel. J Plant Nutr 3:345–356.
Welch RM (1995) Micronutrient nutrition of plants. Crit Rev Plant Sci 14:49–82.
Weng LP, Wolthoorn A, Lexmond TM, Temminghoff EJM, van Riemsdijk WH (2004) Understanding the effects of soil characteristics on phytotoxicity and bioavailability of nickel using speciation models. Environ Sci Technol 38:156–162.
White MC, Baker FD, Chaney RL, Decker AM (1981) Metal complexation in xylem fluid: I. Chemical composition of tomato and soybean stem exudates. Plant Physiol 67:292–300.
Willaert G, Verloo M (1988) Biological effects of nickel species and their determination in plant and soil. Plant Soil 107:285–292.
Wilson MJ, Kordybach BM (2000) Soil Quality, Sustainable Agriculture and Environmental Security in Central and Eastern Europe. Springer, The Netherlands.
Wolfram L, Friedrich B, Eitinger T (1995) The *Alcaligenes eutrophus* protein HoxN mediates nickel transport in *Escherichia coli.* J Bacteriol 177:1840–1843.

Won HS, Lee BJ (2004) Nickel-binding properties of the C-terminal tail peptide of *Bacillus pasteurii* UreE. J Biochem 136:635–641.
Wong MH, Bradshaw AD (1982) A comparison of the toxicity of heavy metals, using root elongation of rye grass, *Lolium perenne*. New Phytol 91:255–261.
Wood BW, Reilly CC (2007) Interaction of nickel and plant disease. In: Datnoff LE, Elmer WH, Huber DM (eds). Mineral Nutrition and Plant Disease. Minneapolis, MN: American Phytopathological Society Press. pp. 217–247.
Wood BW, Reilly CC, Nyczepir AP (2004) Mouse-ear of pecan: A nickel deficiency. Hort Sci 39:1238–1242.
Wood BW, Reilly CC, Nyczepir AP (2006) Field deficiency of nickel in trees: Symptoms and causes. Acta Hort 721:83–98.
Wright DA, Welbourn P (2002) Environmental Toxicology. Cambridge University Press.
Yadav R, Aery NC (2002) Effect of nickel on the growth performance of wheat. Plant Arch 2:133–135.
Yamauchi D (2003) Regulation of gene expression of a cysteine proteinase, EP-C1, by a VIVIPAROUS1-like factor from common bean. Plant Cell Physiol 44:649–652.
Yang J, Zhang J, Wang Z, Zhu Q (2001) Activities of starch hydrolytic enzymes and sucrose-phosphate synthase in the stems of rice subjected to water stress during grain filling. J Exp Bot 52:2169–2179.
Yang X, Baligar VC, Martens DC, Clark RB (1996) Plant tolerance to nickel toxicity: I. Influx, transport, and accumulation of nickel in four species. J Plant Nutr 19:73–85.
Yossef RA, Hegazy MNA, Abdel-Fattah A (1998) Micronutrients in corn plants as affected by the addition N with Ni or Cd. Egypt J Soil Sci 3:427–437.
Youn HD, Kim EJ, Roe JH, Hah YC, Kang SO (1996a) A novel nickel-containing superoxide dismutase from *Streptomyces* spp. Biochem J 318:889–896.
Youn HD, Youn H, Lee JW, Yim YI, Lee JK, Hah YC Kang SO, 1996b. Unique isoenzymes of superoxide dismutase in *Streptomyces griseus*. Arch Biochem Biophys 334:341–348.
Zhang FQ, Wang YS, Lou ZP, Dong JD (2007) Effect of heavy metal stress on antioxidative enzymes and lipid peroxidation in leaves and roots of two mangrove plant seedlings (*Kandelia candel* and *Bruguiera gymnorrhiza*). Chemosphere 67:44–50.
Zhang M, Zhou C, Huang C (2006) Relationship between extractable metals in acid soils and metals taken up by tea plants. Comm Soil Sci Plant Anal 37:347–361.
Zhao F, McGrath SP, Dunham SJ (1999) Factors affecting the solubility of zinc, cadmium, copper and nickel in sewage sludge amended soils. In: Proceedings of Fifth International Conference on the Biogeochemistry of Trace Metals, Vienna, Pp.270–271.
Zia S, Khan MA (2004) Effect of light, salinity, and temperature on seed germination of *Limonium stocksii*. Can J Bot 82:151–157.
Zlesak DC (2007) Factors affecting seed germination of *Heliopsis helianthoides* (L.) Sweet. Seed Sci Technol 35:577–594.

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