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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.

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 ever-increasing media as well as scientific attention: bioterrorism and global warming.

Unfortunately, these very worrisome issues are now superimposed on the already extensive list of ongoing environmental challenges.

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

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

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

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

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

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

Summerfield, NC, USA David M. Whitacre

Contents

Environmental Applications of Chitosan and Its Derivatives

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1 Introduction

Seafood processing waste is a potentially rich source of several useful products including chitin (Meanwell and Shama 2008), and has long been generated in large tonnages worldwide (Chang et al. 2007). Chitin is economical and is the second most abundant bio-waste material after cellulose (Shahidi et al. 1999). Annual worldwide chitin production from arthropods (e.g., crustaceans and insects), molluscs (e.g., squid and cuttlefish) and fungi is estimated at about 100×10^9 t (Tharanathan and Kittur 2003). A steady supply of chitinous waste materials from the seafood processing industry has been the major source of commercial products such as chitin and chitosan (Hayes 2012). The increasing consumption of krill oil and mushrooms has also been an additional source for commercial chitin (Nicol and Hosie 1993; Vetter 2007).

Chitin is a polysaccharide compound dominated by hydroxyl (–OH) and amide (R–CO–NH_2) groups. Chitosan is a by-product of the alkaline deacetylation of chitin, wherein the amide group in chitin is hydrolyzed to a primary amine group (R–NH_2) to produce chitosan. Unlike chitin, chitosan is soluble in acidic solution, but precipitates into solids at a higher pH. Treating chitosan solution at higher pH has been used to transform it into various physical forms (i.e., membranes, nanoparticles, nanofibres, etc.). Even though the high –NH_2 content of chitosan gives the molecule an antimicrobial property (Rabea et al. 2003), it is non-toxic to plants, animals or humans (Baldrick 2010; Kean and Thanou 2010). This lack of toxicity, coupled with its rapid degradability, makes chitosan suitable for several environmental and agricultural uses (Uthairatanakij et al. 2007). Some applications of chitosan include drug delivery in the human gastrointestinal tract (Cook et al. 2013), a role in food processing (Dutta et al. 2009; Romanazzi et al. 2012), biomedical use (Dash et al. 2011; Jayakumar et al. 2010; Khor and Lim 2003; Kim et al. 2007; Muzzarelli 2009), an ingredient in cosmetics (Desbrieres et al. 2010), enzyme immobilization (Krajewska 2004), serving as a heterogeneous catalyst (Guibal 2005), a sorbent for organic and inorganic contaminants (Guibal et al. 2006; Wan Ngah et al. 2011; Wu et al. 2010; Yong et al. 2012), a component of antimicrobial products (Kong et al. 2010), and as an agent to help recover uranium (Muzzarelli 2011).

Soil and water pollution by organic and inorganic contaminants, including metal (loid)s, is of growing concern, because of their potential detrimental effects on human health and the environment (Adriano 2001). With the exception of As, Cr, Hg and Se, most metal(loid)s do not undergo biological or chemical transformation and therefore persist in soils for long periods (Aucott et al. 2010; Bolan et al. 2014). Several methods have been employed to remediate wastewater that is contaminated with toxic metal(loid)s, including chemical precipitation, electrodeposition, ion exchange, membrane separation, and sorption (Geremias et al. 2003). However, these methods are limited by high operational costs (Demirbas 2008) and/or inefficiency for remediation of some toxic metals that exist at trace levels (Juang and Shao 2002).

Renewable chitinous waste materials derived from plants or animals may be a cost-effective approach to remediate wastewater (Niu and Volesky 2007). Crab shells have effectively been used to decontaminate metals (e.g., Pb, Cd, Cu, and Cr) in wastewater (An et al. 2001; Kim 2003). Fungus mycelium biomass has recently emerged as a promising new source of chitinous material to potentially remediate contaminated wastewater (Kamari et al. 2011a, b; Tay et al. 2011a, b, 2012). The commercial value of employing chitosan for environmental applications may be enhanced considerably by modifying its structure and chemical functionalization. For example, by applying a crosslinking process on chitosan both its resistance to acidic solubilization (Hsien and Rorrer 1995) and to microbial degradation are enhanced (Yamamoto and Amaike 1997).

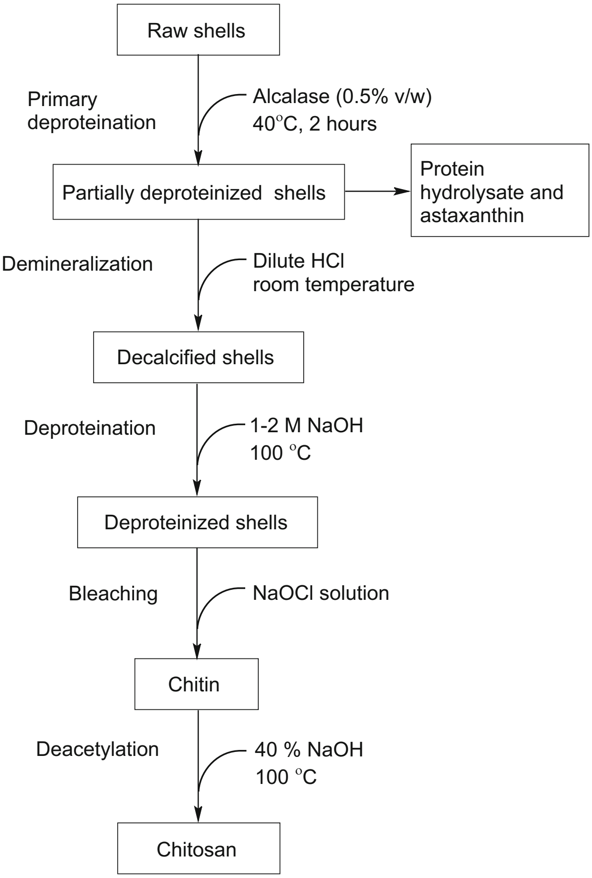
Pillai et al. (2009) and Roberts (2008) have previously described the general applications to which chitin and chitosan have been put. However, to the best of our knowledge, the environmental applications of chitosan or its derivatives have not previously been reviewed in detail. Therefore, it is our aim in this review to close this gap by addressing the latest developments made with chitosan for environmental applications. In particular, we emphasize chitosan's use for mitigating heavy metals and organic contaminants in soil and wastewater. In addition, we cover chitosan's general properties, its limitations and prospects for chemically modifying it in ways that enhances its performance and utility for environmental applications.

2 Production and Properties of Chitosan

2.1 *Production of Chitosan*

Chitosan is derived from chitin through a deacetylation process, whereby, the acetamide group is hydrolyzed to produce acetate ions and an $–NH_2$ group (Fig. 1). Production of chitosan from raw shells involves four major steps, viz., deproteination, demineralization, bleaching and deacetylation. Chitin is produced by removing protein and calcium carbonate from the raw shells and cuticles via

Fig. 1 Process for producing chitosan from shrimp shell wastes



deproteination and demineralization processes, respectively. The amount of chemicals and reaction time needed to produce chitosan depends on the origin of the raw chitinous materials. Harsher reaction conditions are required to demineralize crab and lobster shells (due to their higher calcium carbonate content) than are needed for prawn shells or squid pen.

Crab shells are deproteinized by incubation in a solution of 1 M sodium hydroxide (NaOH) at 90 °C for 24 h, followed by demineralization in 1 M hydrochloric acid (HCl) for 24 h (Peniche et al. 2008). Usually, a complete demineralization of shrimp shells at room temperature is achieved within 15 min by using 0.25 M HCl at a liquor ratio of 40:1. The amount of HCl required for demineralization must be accurately calculated (i.e., from the calcium carbonate content of prawn shells) to minimize the hydrolysis of chitin molecules by residual HCl. The subsequent deproteination stage is relatively mild, and involves incubation in 1 M NaOH at 70 °C for 24 h. A lower NaOH concentration (0.3 M) has also been used to deproteinize various marine chitinous materials at 80–85 °C; however, using NaOH during deproteination may cause browning of chitinous materials

(Rhazi et al. 2000). Carotenoids in prawn or shrimp shell may also impart an unwanted reddish color to chitin. Hence, an optional bleaching process may be added prior to deacetylation to decolorize chitin. A simple, but less effective decoloration process is to irradiate chitin with sunlight (Youn et al. 2007). Traditionally, organic solvents (e.g., acetone, ethanol, chloroform and ethyl acetate) and oxidizing agents (e.g., sodium hypochlorite and hydrogen peroxide) have been used to decolorize chitin. Although oxidizing agents are more effective than organic solvents for bleaching chitin, they may decrease the molecular weight of chitin via oxidative depolymerization.

The final step for chitosan production is deacetylation. To date, most chitin deacetylation processing has been conducted through a thermo-chemical reaction, using a 40% (wt/wt) NaOH or KOH solution at 100 °C. The degree of deacetylation of chitosan increases with increasing NaOH concentration, temperature and heating time during the deacetylation process (Methacanon et al. 2003; Santhosh et al. 2010). However, excessive use of NaOH and energy in the thermo-chemical deacetylation process makes it unsustainable due to high cost and its potential adverse impact on the environment. Sodium ions in wastewater from the thermo-chemical deacetylation process may adversely impact soil quality. In addition, the harsh conditions in the thermo-chemical process may degrade chitosan and thereby decrease its molecular weight. To reduce reaction time, temperature and consumption of NaOH, a thermo-chemical deacetylation process has been utilized that employs either microwave (Goycoolea et al. 1997), gamma ray (Tahtat et al. 2007) or ultrasound irradiation treatments (Delezuk et al. 2011). However, such methods still consume a significant amount of energy and NaOH, and may not be economically viable, given that additional energy and wastewater treatment further increase chitosan production costs.

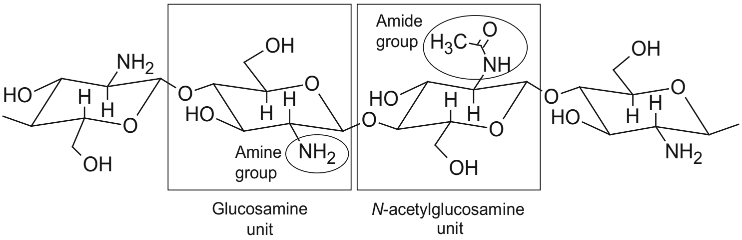
Recently, enzymatic and microbiological fermentation processes have emerged as a promising alternative pathway to produce chitosan economically. In these, the thermo-chemical process is replaced with either enzymatic reactions or fermentation with enzyme-producing microbes (Wang et al. 2011). The latter process utilizes acidic by-products of cheese whey fermentation (i.e., lactic acid and acetic acid) as substitutes for HCl in the demineralization process (Mahmoud et al. 2007). Lactic acid bacteria have also been used to demineralize crab shells (Jung et al. 2005) and prawn shells (Rao and Stevens 2005). A protease enzyme, which is co-produced by lactic-acid-producing bacteria during fermentation, may further deproteinize chitin. Fungi (i.e., *Aspergillus niger*) have also been used to deproteinize chitin (Mahmoud et al. 2007). The advantages of using enzymatic and microbiological fermentation processes over the conventional thermo-chemical deacetylation process include lower energy consumption, reduced production of wastewater and reduced hydrolysis of the chitosan structure. The milder reaction conditions of the enzymatic and microbiological processes allow preservation of protein by-product, which can then be processed as a value-added food product for human (Coral-Hinostroza and Bjerkeng 2002) or animal consumption (Hirano et al. 1990).

Another small-scale chitosan production method has been employed by utilizing fungal proteolytic enzymes to demineralize and deproteinize prawn shell powder (Teng et al. 2001). Compared to the conventional chitosan production methods, this approach offers two advantages: (a) it provides an additional source of chitin from the mycelium biomass of the fungi, and (b) it utilizes protein in prawn shell waste as a source of nitrogen for fungal growth.

The enzymatic deacetylation process of chitin utilizes chitin deacetylase, which is produced by fungi (e.g., *Mucor rouxii*, *Saccharomyces cerevisiae*, etc.), marine bacteria (i.e., *Vibrionaceae cholerae*) and some insects. A powdered sample of chitin is treated with chitin deacetylase or is fermented with chitin-deacetylase-secreting-microbes. The enzymatic process yields chitosan that possesses a low degree of deacetylation and depolymerization (Tsigos et al. 2000). Araki and Ito (1975) reported that enzymatic deacetylation alone of glycol chitin released about 30% of acetyl groups. However, compared to the thermo-chemical process, the enzymatic deacetylation efficiency of the crystalline chitin substrate to produce partially-deacetylated-chitin is still low. Hence, further treatment of partially-deacetylated-chitin is necessary to obtain chitosan that has a high degree of deacetylation ($\geq$60%). Moreover, the enzymatic process produces a mixture of partially-deacetylated-chitin, calcium salts and hydrolyzed protein, which needs further isolation. In reality, enzymatic deacetylation may be more practical when applied to the more water soluble amorphous chitin substrate (i.e., partially deacetylated chitin, chitin oligomers or glycol chitin). Currently, it is still not economically feasible to deacetylate chitin by using enzymatic processes (Zhao et al. 2010).

2.2 Properties of Chitosan

In general, chitin and chitosan are polysaccharides that have multiple hydroxyl (–OH) and amide (R–CO–NH_2) or amine (R–NH_2) functional groups. Chitin and chitosan are both co-polymers of glucosamine and *N*-acetylglucosamine units with varying molecular weights, depending on the extent of degradation of chitin and chitosan polymer. Chitin primarily consists of *N*-acetylglucosamine units with >40% amide groups, whereas chitosan is mostly glucosamine with $\geq$60% amine groups (Roberts 1992). The structures of chitin and chitosan are presented in Fig. 2. The physical and chemical properties of chitosan depend on its molecular weight and degree of deacetylation (Wang et al. 2006). The extensive number of –NH_2 and –OH groups causes intermolecular interactions, such as hydrogen bonds and other non-specific interactions between chitosan molecules. Compared to chitin, chitosan is more soluble in acidic solution. Bulk chitosan molecules dissolve in monoprotic acids such as formic, acetic, hydrochloric and nitric acids. In acidic solution, chitosan becomes positively charged, when an –NH_2 group accepts a proton to form a protonated amine group (–NH_3^+). Consequently, the positively charged chitosan particles are separated through repulsive force and become suspended in aqueous acidic solution. The solubility of chitosan increases with the increase in



Legend: chitin (>40% amide groups); chitosan (≥60% amine groups)

Fig. 2 Structures for chitin and chitosan

–NH_2 groups (Sannan et al. 1976). An acidic chitosan solution, with a concentration of up to 15 g/L has been successfully prepared using 0.2 M acetic acid (Barreiro-Iglesias et al. 2005).

The acidic chitosan solution can be precipitated by a phase inversion process, wherein the solution pH is increased to >6.5 by adding alkali solution (e.g., NaOH, KOH or NH_4OH). The hydroxide ions deprotonate the –NH_3^+ group of the dissolved chitosan to form a chitosan precipitate. This technique has been applied to produce porous hydrogel (Dang et al. 2011), beads (Rorrer et al. 1993), fibers (Suzuki 1993) (1992, patent appl date), membranes (Uragami et al. 1983), and sponges (Denkbaş and Odabaşi 2000). The acidic chitosan solution can be converted to semi-solid phase through a gelation process. Chitosan ionic gel has been produced by mixing chitosan with polyprotic acids such as sulfuric and phosphoric acids. Larger polycarboxylates (e.g., oxalate, malonate, succinate, citrate, and alginate) are also used to engineer chitosan ionic gels (Hamdine et al. 2005). The ionic gelation mechanism relies on forming a strong intermolecular interaction (i.e., ionic bonds) between –NH_3^+ in chitosan and the anions of polyprotic acids.

Chitosan is an excellent sorbent for trace metals due to the excess of –OH and –NH_2 groups that it possesses, and the high flexibility of its structure (Miretzky and Cirelli 2009). Sorption of trace metals by chitosan is selective and independent of the size and hardness of metal ions, or the physical form of chitosan (e.g., film, powder and solution) (Rhazi et al. 2002). The metal affinity sequence of the chitosan film was in the order: Cu(II) ≥ Hg(II) > Zn(II) > Cd(II) > Ni(II) > Co(II) ~ Ca (II) for divalent cations; and Eu (III) ≥ Nd (III) > Pr(III) for trivalent cations (Muzzarelli 1973). Kim (2004) reported a similar order of metal affinity sequences for chitosan: Cu(II) > Fe(II) > Zn(II) > Cd(II). Vold et al. (2003) used the selectivity coefficient, $k^{B}{}_{A}$ (Eq) to compare binding of two different metal ions (represented by A and B) to chitosan:

$$k^{B}{}_{A} = X_B \times C_A / X_A \times C_B$$

where X_A and X_B are the mole fractions of A and B, respectively ($X_A + X_B = 1$); and C_A and C_B are the molar concentrations for A and B at equilibrium. Chitosan showed greater selectivity for Cu(II) with $k^{B}{}_{A}$ values ranging from 100 to 1,000, when compared to other metals such as Zn(II), Cd(II) and Ni(II) (Vold et al. 2003). Initially, it was proposed that the interaction between metal ions and chitosan was due to ion exchange, sorption and chelation (Muzzarelli et al. 1986). However, Vold et al. (2003) suggested that the metal sorption selectivity of chitosan did not depend upon the ionic strength and pH of the solution. Sorption of uranyl ions by chitosan is independent of pH (Piron and Domard 1998), which indicates that the sorption mechanism of metal ions with chitosan is inclined to complexation, rather than electrostatic attraction. Metal ions are coordinated to the $–NH_2$ groups of chitosan to form a pendant-like metal-chitosan complex (Lü et al. 2008). The stability of metal-chitosan complexes is further enhanced by chelation, whereby the metal ion is coordinated to two –OH groups and two $–NH_2$ groups to form a bridge-like metal-chitosan complex (Klepka et al. 2008).

Solubilization of chitosan using strong and concentrated acid at high temperature may cause glycosidic hydrolysis (Sklyar et al. 1981), producing smaller chitosan fragments having low molecular weight (i.e., chito-oligosaccharide and glucosamine). Thermo-chemical deacetylation also decreases the average molecular weight of chitosan from 1.03×10^6–2.5×10^6 g/mol to 1×10^5–5×10^5 g/mol (Austin et al. 1981). Chito-oligosaccharide and glucosamine have low viscosity and are more soluble than chitosan at relatively neutral pH conditions. Both chito-oligosaccharide and glucosamine are bioactive (e.g., anti-microbial, anti-inflammatory, anti-oxidant, etc.) (Xia et al. 2011), and have applications in medicine (Hirano 2000), textiles (Lim and Hudson 2003) and food preservation (Dutta et al. 2009). The $–NH_3^+$ group of dissolved chitosan particles bind with the negatively charged cell walls of microbes, eventually causing leakage of their intracellular constituents (Rabea et al. 2003). However, such leakage is not of concern because chitosan is not persistent in the biosphere and is biodegraded to chito-oligosaccharide and finally to carbon dioxide (Ratajska and Boryniec 1998). In another study, an increase in the degree of deacetylation of chitosan reduced the *in vivo* (i.e., rats) and *in vitro* (i.e., egg white lysozyme) biodegradation rates of chitosan film (Tomihata and Ikada 1997).

Chitosan is non-toxic to higher organisms such as animals and humans. The median lethal dose (LD_{50}) of orally administered chitosan for rats and mice is >1,500 mg/kg and 16,000 mg/kg, respectively. The LD_{50} of orally administered chito-oligosaccharide is >10,000 mg/kg for mice (Baldrick 2010). However, intravenous administration of a high-dose chitosan (50 mg/kg) may be lethal due to blood cell aggregation (Kean and Thanou 2010). Dogs injected with 200 mg/kg of chitosan suffered severe haemorrhagic pneumonia and died after 8 days (Minami et al. 1996). The oral LD_{50} of chitosan for humans is estimated to be 1,330 mg/kg (Prajapati 2009). Even with the administration of high oral doses (6.75 g daily per human subject for 84 days), no clinically significant adverse symptoms were observed other than mild to transitory nausea and constipation (Baldrick 2010). However, chitosan may possibly be allergenic to humans. Marine chitosan (sourced from crabs and prawns) is likely to cause allergic reactions from the presence of antigens that trigger release of immunoglobin E antibodies (IgE). However, Muzzarelli (2010) suggested that neither chitin nor chitosan is allergenic to humans. To date, there has been no direct evidence that marine chitosan products cause allergic reactions in humans. Results from two pilot studies on shrimp-derived glucosamine supplements (Gray et al. 2004) and a chitosan bandage (Waibel et al. 2011) produced no evidence of allergic reaction in human subjects. Rather, chitosan has been a popular weight management supplement for animals and humans (Walsh et al. 2013), which further suggests that chitosan is safe for environmental applications.

2.3 Chitosan Modification

Attempts have been made to chemically modify chitosan to overcome the issue of its acid instability (due to solubility). Such modification would not change the fundamental skeleton of chitosan, but rather would bring new or improved properties. Guibal et al. (1998) found that enhancing the solubility of chitosan in acidic wastewater (e.g., due to acidic mine drainage) results in low porosity, low hydraulic conductivity and low permeability that can clog pore structures in a wastewater treatment. Acidic wastewater may also cause dissolution of chitosan, resulting in a loss of mass that could lead to structural failure of the chitosan sorbent (Oshita et al. 2002). Hence, the structure and functional groups of chitosan must be optimized to enhance its stability and metal sorption capacity.

Chitosan's instability under acidic conditions has adversely affected its practical use, particularly when using an acidic eluent to regenerate spent sorbent. Introducing crosslinks to the chitosan structure has successfully enhanced its durability in acidic environments. Crosslinking of chitosan was achieved by using various crosslinking agents, such as glutaraldehyde (GA) (Guibal et al. 1998), epichlorohydrin (ECH) (Baba et al. 1994), ethylene glycol diglycidyl ether (EGDE) (Kamari et al. 2009), and hexamethylene diisocyanate (HMDIC) (Choudhari et al. 2007) (Fig. 3; see Table 1 for a list of abbreviations and acronyms used in this paper). The enhanced durability from crosslinked chitosan was attributed to its enhanced resistance to structural changes under hydrated or acidic conditions (Berger et al. 2004). Hsien and Rorrer (1995) reported that the surface area of chitosan beads increased after crosslinking, although, crosslinking may block sorption sites (i.e., –NH_2 groups) and cause a significant decrease of metal sorption capacity (Milot et al. 1998). For example, Cu (II) sorption capacity of GA-crosslinked chitosan was lower than that of non-crosslinked chitosan (Osifo et al. 2008). A crosslinking process that prevents loss of –NH_2 groups in chitosan is crucial to maintain high sorption capacity for metal ions. Shin et al. (2011) used benzaldehyde to protect –NH_2 groups in chitosan, prior to crosslinking with ECH. The resulting ECH crosslinks were formed at the C_6 –OH group in chitosan. Subsequent treatment of the crosslinked chitosan with ethanol hydrochloride decomposed the Schiff base derivative to –NH_2 groups, thereby, maintaining the –NH_2 group content for metal-ion binding (Sahin et al. 2011).

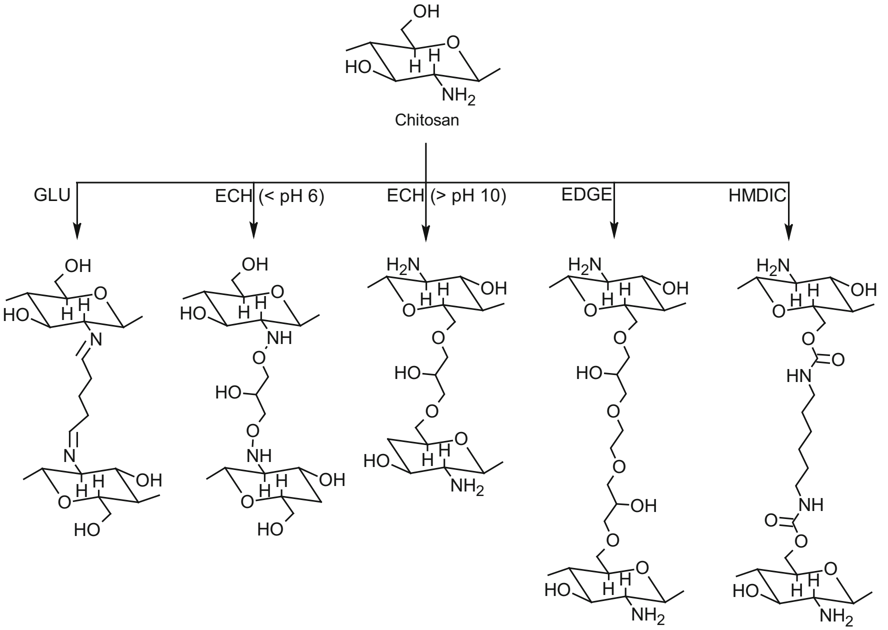


Fig. 3 Crosslinking of chitosan with glutaraldehyde (GA), epichlorohydrin (ECH), ethylene glycol diglycidyl ether (EDGE) and hexamethylene diisocyanate (HMDIC)

The stability of complexed metals in chitosan's structure also affects metal sorption capacity. The Hard Soft Acid Base (HSAB) theory (Pearson 1963) predicts that the $–NH_2$ and –OH groups in chitosan are borderline and hard Lewis bases, respectively. However, any sorbed soft Lewis acids (e.g., Cd(II), Pd(II), Pt(II), etc.) may produce unstable metal-chitosan complexes that decrease the sorption capacity for these metals. To reverse this instability, chitosan has been chemically modified to introduce soft Lewis bases (e.g., sulfide, dithiocarbamate, thiourea, etc.). For example, grafting of chitosan with thiourea derivatives increased sorption capacities for Pt(II), Pd(II), Au(III) (Bratskaya et al. 2011), and Cd(II) (Yong et al. 2013). Similarly, grafting increased the number of negative charged functional groups for metal complexation. Chloroacetic acid has been used to introduce carboxymethyl groups ($–CH_2COOH$) to $–NH_2$ or –OH groups to increase the number of chitosan metal-binding sites (Muzzarelli and Tanfani 1982). Polyprotic acid (i.e., citric acid) has been grafted to create more negatively charged binding sites for metals to improve Pb(II) sorption capacity (Li et al. 2010).

Chitosan has been modified to enhance sorption selectivity for specific metal ions, whereby the intended metal ion is used as a template to create specific cavities in the chitosan structure. The metal templates are removed from the chitosan structure by rinsing with diluted acid solution, leaving specific cavities that are only accessible to the intended metal ions. For example, Ga(III) (Inoue et al. 1996), Cu(II) (Cao et al. 2004) and Pb(II) (Sun et al. 2006; Wang et al. 2010a) ions have

Table 1 List of abbreviations and acronyms used in this paper

AChE	Acetylcholinesterase
ALP	Alkaline phosphatase
2,4-D	2,4-Dichlorophenoxyacetic acid
2,4,5-T	2,4,5-Trichlorophenoxyacetic acid
BPA	Bisphenol A
CuCH	Copper-chitosan
DCB	2,2′-Dichlorobiphenyl
DCP	2,4-Dichlorophenol
DD	Degree of deacetylation
DTPA	Diethylene triamine penta acetic acid
ECH	Epichlorohydrin
EDDS	Ethylenediamine-*N*, *N*′-disuccinic acid
EDGE	Ethylene glycol diglycidyl ether
EDTA	Ethylene diamine tetra acetic acid
GA	Glutaraldehyde
HDACS	Hexamethylene-1, 6-diaminocarboxysulfonate
HMDIC	Hexamethylene diisocyanate
HRP	Horseradish peroxidise
HSAB	Hard soft acid base
LDH	Layered double hydroxides
MCM-chitosan	Monocarboxymethylated-chitosan
MWNT	Multiwall carbon nanotube
NTA	Nitrilotriacetic acid
OPH	Organophosphorous hydrolase
PAH	Polyaromatic hydrocarbon
POME	Palm oil mill effluent
PPO	Polyphenol peroxidise
PRB	Permeable reactive barrier
PTAA	Polythiophene-3-acetic acid
SA	Salicylic acid
SCTA-I	Thiol-modified chitosan
SPR	Surface plasmon resonance
TCE	Trichloroethylene
TCP	2,4,6-Trichlorophenol
TPP	Tripolyphosphate
TYR	Tyrosinase
VOME	Vegetable oil mill effluent

been used as templates to form metal-specific chitosan sorbents for wastewater remediation. Cao et al. (2001) further introduced GA crosslinks to the structure of Cu(II)-templated chitosan resin, producing stable chitosan resins in acidic solution that also possess high sorption capacity for Cu(II), Ni(II) and Co(II) ions.

Chitosan has been blended with other materials to form composites that enhance sorption properties (Wan Ngah et al. 2011). Materials with negative surface charge are generally used in chitosan composites, not only to increase binding sites for sorption of metal ions, but also to form a stable ionic composite with the $–NH_3^+$ group of chitosan. Alginic acid was blended with chitosan and then crosslinked with GA to produce sorbent beads (Gotoh et al. 2004). Chitosan was also used to coat a montmorillonite clay sorbent for remediation of tungsten (W) from drinking water (Gecol et al. 2006).

3 Chitosan Applications

Chitosan is generally regarded as a non-toxic and biocompatible material for humans and animals, and has been widely used in the fields of agriculture, pharmaceuticals, and industrial food processing. Chitosan has also been used for several environmental applications, including remediation of both organic (e.g., colored dyes, residual oil/solid from palm oil mill effluent) and inorganic contaminants (e.g., toxic trace metal(loid)s) from wastewater and soil. However, there are several challenges when using chitosan for environmental applications, particularly in terms of maintaining its stability and efficacy for remediating contaminated wastewater and soil.

3.1 Remediation of Inorganic Contaminants in Aqueous Systems

Chitosan has been evaluated for remediating heavy metals, such as Cu (II) (Ng et al. 2002), Pb(II) (Ng et al. 2003), Hg(II) (Shafaei et al. 2007), Cd (II) (Rorrer et al. 1993) and Zn(II) (Karthikeyan et al. 2004). It is regarded as one of the best natural chelators for trace metals (Chui et al. 1996). The chitosan molecule is sufficiently flexible to form a helical structure around metal ions (Ogawa et al. 1993), which forms multiple coordination bonds with each ion (Wu et al. 2010). Metal sorption can be augmented by physically modifying the chitosan sorbent. For example, a phase inversion process can be used to produce porous chitosan beads, which may lead to increased metal sorption capacity, possibly from increased surface area (Rorrer et al. 1993).

Sorption of metal ions by chitosan is usually optimum at pH values higher than chitosan's p*K*a value (>6.5) (Guzman et al. 2002; Navarro et al. 2003). A high sorption pH value (<6.5) induces deprotonation of $–NH_3^+$ to $–NH_2$ groups, thus enabling coordination of metal cations and increasing sorption capacity (Jeon and Höll 2003). Physical optimization of chitosan has also been conducted by mixing it with negatively charged substances. Qi and Xu (2004) precipitated chitosan-tripolyphosphate composite nanoparticles (CS-TPP) (mean sizes = 40–100 nm) from chitosan hydroacetate solution using aqueous sodium tripolyphosphate solution. The CS-TPP was found to have high Pb(II) sorption capacity (398 mg/g), owing to the low crystalinity of CS-TPP and the presence of the TPP ion (Qi and Xu 2004).

Chitosan has been used to economically remediate wastewater resulting from electroplating activities (Coughlin et al. 2006), and to recover precious metals from mining wastewater (Benavente et al. 2011). The abundance of $–NH_2$ groups in chitosan allows it to successfully remediate toxic metal oxyanions (e.g., chromate and arsenate) from wastewater. Metal oxyanions are adsorbed to $–NH_3^+$ ions on chitosan through an ion exchange mechanism (Navarro et al. 2003), although the arsenate ion sorption from aqueous systems using chitosan beads was low (<12 mg/g) (Chassary et al. 2004). Mixing chitosan with montmorillonite (Assaad et al. 2007) and alginate (Wan Ngah and Fatinathan 2010) enhanced sorption of metal ions from wastewater. The sorption mechanism involved metal chelation by $–NH_2$ groups, as well as coordination/coagulation of metal ions by polar groups of montmorillonite (i.e., silanol and aluminol) and alginate (i.e., carboxylate) (Assaad et al. 2007; Qi and Xu 2004; Wan Ngah and Fatinathan 2010).

Modifying chitosan with molybdate ions enhanced the sorption capacity of arsenate ions from complexation of arsenate ions to the modified chitosan (Dambies et al. 2002). Similarly, composites of Fe(III) and chitosan were synthesized to enhance sorption of arsenate ions (Gupta et al. 2009; Gupta and Sankararamakrishnan 2010). Pulverized Fe(III)-chitosan nanocomposite was chemically reduced to produce zero-valent iron, which further enhanced sorption of arsenate ions. Arsenate ions were reduced by zero-valent iron to arsenite ions, and were subsequently complexed by the oxidized iron (Fe(III)) (Gupta et al. 2012). Similarly, remediation of chromate-contaminated wastewater may also involve a reduction mechanism. At low pH (4.5), sorbed chromate ions may be partially reduced by chitosan (Dambies et al. 2001). The reduction of chromate ions was possible because of their high reduction potential ($E_o = (-1.33$ V) under acidic conditions.

These observations have inspired numerous chitosan modifications, specifically designed to reduce chromate ions in wastewater, such as introduction of xanthate to chitosan (Sankararamakrishnan et al. 2006), synthesis of chitosan nanoparticles with zero-valent iron (Geng et al. 2009a, b) and entrapment of zero-valent iron nanoparticles in chitosan beads (Liu et al. 2010). GA-crosslinked chitosan also enhanced reduction of chromate ions, possibly from the presence of free aldehyde groups of GA (Vieira et al. 2011). The reduction process helps alleviate the pollution impact by converting toxic Cr(VI) ions to relatively less toxic Cr(III) ions.

Chitosan has also been used to remediate water resources contaminated with non-metal anions (i.e., nitrates, nitrites, phosphates and fluoride). Similar to the mechanism by which nitrate and orthophosphate are sorbed, the $–NH_3^+$ ions in chitosan also sorb fluoride ions. Such sorption would be enhanced at low pH, where the amount of $–NH_3^+$ groups is high (Chatterjee and Woo 2009). Even though acidic conditions increase the amount of $–NH_3^+$ groups, fluoride ions may form hydrofluoride vapor at pH levels <5, which would decrease fluoride ion sorption from wastewater (Miretzky and Cirelli 2011). However, as chitosan is physically unstable at low pH values, sorbent made from chitosan would normally be stabilized by treatment with a crosslinking agent (e.g., GA (Jaafari et al. 2004) or ECH (Chatterjee et al. 2009)). Huang et al. (2012) demonstrated that the protonated form of the GA-crosslinked chitosan particle (CCP) enhanced fluoride ion sorption capacity and stability of chitosan as compared to the deprotonated form of GA-crosslinked chitosan. The enhanced stability of CCP in acid enables regeneration using a 0.1 M HCl solution (Huang et al. 2012). Lanthanum ion (La(III)) has also been chelated to the $–NH_2$ groups of chitosan flakes (Rayalu et al. 2007) and chitosan beads (Bansiwal et al. 2009) that successfully enhanced fluoride sorption.

Chitosan has been used to immobilize symbiotic microorganisms for bioremediation of orthophosphate and nitrate ions. For example, chitosan was utilized to form aggregates with microalgae *Phormidium* (Chitosan: *Phormidium* = 1:2, dry wt basis) to remove 98% of nitrogenous compounds and 88% of phosphate compounds from a secondary effluent during a 24 h period (De La Noue and Proulx 1988). The reduction of orthophosphate and nitrate ion levels in effluent resulted from direct assimilation of orthophosphate and inorganic nitrogen ions by *Phormidium*, while the presence of chitosan protected the algae from abrasion.

3.2 Remediation of Metal-Contaminated Soils and Sediments

Remediation methods for metal-contaminated soils can be roughly classified into three types: physical, chemical and biological (including phytoremediation) (Zhou and Song 2004). Metal-contaminated soil can be remediated with chitosan either by (1) active removal of metal ions from the contaminated soil through extraction of metal complexes, or (2) immobilization of metal ions (Peng et al. 2009). Generally, treatment of contaminated soil with organic matter (i.e., chitin and chitosan) helps immobilize metals by precipitation, sorption or complexation (Mench et al. 1994; Park et al. 2011). Addition of acid-dissolved, positively charged chitosan changes the physicochemical properties of soil, eventually immobilizing heavy metals therein. Chitosan-coated sand, which had been pre-treated with NaOH, improved the sorption capacity of Cu(II) and reduced its leachability (Wan et al. 2004). This shows that the metal-retention properties of chitosan is only significant when the $–NH_2$ group is not protonated. Conversely, in another study, a sand-packed column had low Cu(II) sorption capacity and high leachable Cu(II) content with the addition of an acidic chitosan solution (Etemadi et al. 2003). Bulk density, cationic exchange capacity (CEC) and pH decreased when an acidic chitosan solution was added to soil, whereas the electrical conductivity (EC) increased (Hu et al. 2006). This appears to be a more realistic scenario, given that Cu and other metal cations tend to sorb onto negative charged surfaces (Srivastava et al. 2005).

Chitosan has been used to treat metal-containing leachate after active remediation of contaminated soil. Bassi et al. (2000) used a citric acid solution as a complexing agent to leach metal ions from contaminated soil. The leachate was then treated with chitosan flakes to remove metal ions. Other complexing agents such as ethylenediamine tetra acetic acid (EDTA), diethylene triamine pentaacetic acid (DTPA) and nitrilotriacetic acid (NTA) were shown to effectively mobilize metal ions from contaminated soil (Barona et al. 1999; Barona and Romero 1996; Elliott and Brown 1989; Saifullah et al. 2010). However, NTA has been reported as a potential carcinogen to humans (Pohanish 2008). Although EDTA and DTPA are themselves relatively non-toxic, their use may cause poisoning by making toxic metals more bioavailable (Barona et al. 2001). As previously mentioned, chitosan is non-toxic to plants, animals and humans (Baldrick 2010; Kean and Thanou 2010), and is therefore a safe complexing agent from the health and safety perspective. Low molecular weight chitosans (i.e., chito-oligosaccharide and glucosamine) produced by acidic hydrolysis or oxidation of chitosan are safe complexing agents for active remediation of contaminated soil. Although chito-oligosaccharide and glucosamine hydrochloride were inferior to EDTA and L-asparaginic-*N*, *N*-diacetic acid (ASDA), in terms of their ability to leach Cu(II) ions from vermiculite (Guo and Inoue 2003), they are non-toxic and do not adversely impact the environment. Chitosan has also been used to extract Cd(II) (Li et al. 2008) and Cr(VI) ions (Li et al. 2009) from contaminated soils. Guo et al. (2009) observed enhanced extraction of Cu(II) and Cd(II) ions with chitosan hydrochloride solution in subsurface soil (14–16 and 24–26 cm) after a 7–14 days incubation period. Cd(II) was better extracted than Cu(II), confirming the high affinity of chitosan for Cu(II) ions (Guo et al. 2009).

Chitosan was used in a permeable reactive wall (Liao et al. 2010) (2009, patent appl date) to remediate metal ions in soil. Chitosan lixiviant liquid was pumped into the soil to mobilize toxic metal ions from the contaminated soil, which were then removed by the permeable reactive wall. Table 2 shows examples of chitosan and its derivatives being used as lixiviants for metal mobilization, thereby demonstrating that they augment metal sorption by plants during phytoremediation of contaminated soil. Monomeric chitosan (glucosamine) forms metal complexes that

Table 2 Chitosan and its derivatives used in the plant uptake of metals from contaminated soil

Chitosan derivatives	Metal	Plant	Reference
Chitosan	Pb(II)	*Zea mays* L.	Liu et al. (2006a)
Chitosan	Pb(II)	*Zea mays* L.	Liu et al. (2006b)
Chitosan	Cu(II) and Cd(II)	*Elsholtzia splendens*	Weng et al. (2005)
Chitosan	Zn(II), Pb(II) and Cd(II)	*Elsholtzia splendens*	Wang et al. (2007)
SCTA-I	Pb(II)	*Zea mays* L.	Yang et al. (2006)
GA-crosslinked chitosan	Cu(II), Zn(II) and Pb(II)	*Lolium perenne* and *Brassica napus*	Kamari et al. (2012)

Note: *SCTA-I* thiol-modified chitosan, *GA* glutaraldehyde increased the availability of metal ions for plant absorption. Addition of chitosan to soil at pH 2 increased desorption of Pb(II) (Liu et al. 2006a), and led to enhanced uptake of Pb(II) by corn (*Zea mays* L.) from contaminated soil (Liu et al. 2006b). With the addition of chitosan, the Pb(II) concentration in plant roots was threefold higher than in the control (without chitosan) (Liu et al. 2006b). Similarly, adding chitosan and ethylene diamine disuccinate (EDDS) helped *Elsholtzia splendens* accumulate Cu(II) and Cd(II) (Weng et al. 2005). Adding chitosan to microbial inocula (Arbuscular mycorrhizal fungi and *Penicillium* sp.) was found to synergistically increase Zn(II), Pb(II) and Cd(II) accumulation in the shoots of *E. splendens*. Weng et al. (2005) attributed this synergistic effect to the mobilization of metal ions by complexation with chitosan. Water-soluble metal-chitosan complexes enabled greater plant absorption, possibly from increased partitioning of metal ions to the shoot tissues of the *E. splendens* plants (Wang et al. 2007).

Modified chitosan has been shown to improve extraction and phytoremediation of metal ions from contaminated soil (Yang et al. 2006). A thiol group was introduced to the $-NH_2$ groups in chitosan to produce thiol-modified chitosan (SCTA-I), which enhanced Pb(II) uptake in corn roots and shoots from a Pb-contaminated soil. The enhanced absorption of Pb(II) possibly resulted from the greater ability of SCTA to extract Pb(II) from soil, compared to citric acid and chitosan (Yang et al. 2006).

Chitosan and its crosslinked derivatives have been used as amendments for immobilizing metals in contaminated soil. The solid form of chitosan can be applied to adsorb metal ions from moist soil, thereby reducing the availability of toxic metal ions to living organisms. Nonetheless, using chitosan alone may not be fully effective for remediating contaminated soil, because of its low affinity for metal ions (i.e., Cd(II), Zn(II), etc.). This issue may be overcome by blending chitosan with materials having different functional groups to form a composite that augments its activity. For example, the chitosan-apatite composite more strongly retains a wide range of metal ions (Knox et al. 2008).

As described previously, stability is a potential issue, when using chitosan to remediate metal ions. Natural degradation may break chitosan down to chito-oligosaccharides (Kwon et al. 2010), and possibly release immobilized metal ions into the environment. Hence, chitosan that employs a crosslinking process may be essential for modulating natural degradation processes that involve chitosan. Kamari et al. (2011a) reported the remediation of metal contaminated soil by using both chitosan and crosslinked chitosan as soil amendments. In these studies, ECH, GA and EDGE were used for crosslinking, and each of these treatments increased the surface area of chitosan. Leaching of the adsorbed metal ions was lower with crosslinked chitosan than for pristine chitosan (Kamari et al. 2011b). Kamari et al. (2012) further reported that the low application rate of chitosan (at 1% wt/wt) enhanced metal sorption by plants, possibly by forming water-soluble metal-chitosan complexes. However, a high application rate of chitosan (at 10% wt/wt) decreased metal sorption by perennial ryegrass (*Lolium perenne*). The metal sorbed by rapeseed (*Brassica napus*) was also decreased from the chitosan amendment, regardless of application rate. High application rates of chitosan or crosslinked chitosan amendments may inhibit formation of the water-soluble metal-chitosan complex, thereby reducing metal sorption by plants.

Microbes have been used with chitosan to improve removal of metal ions from contaminated soil. Cuero (1996) enhanced the removal of Zn(II) and Cu(II) ions from contaminated soil by using a mixture of bacteria (*Bacillus subtilis*) and chitosan in solution. Ideally, microbes immobilized with chitosan show greater stability, remediation efficiency, and regenerability (de-Bashan LE and Bashan 2010). A composite that consists of chitosan, alginate and polyvinyl alcohol was crosslinked with ECH to immobilize inactive *Saccharomycetes pombe* 806 yeast biomass for sorbing Cd(II) from soil leachate (Lin and Lin 2005). The concentration of Cd(II) in soil leachate decreased from 3.91 to 0.269 mg/L, when using 0.3 g of the yeast-immobilized-chitosan beads.

3.3 Remediation of Organic Contaminants

Soil and wastewater that are contaminated with organic contaminants can be remediated with chitosan, although the success is somewhat dependent on the characteristics of the contaminants. In Table 3, we summarize studies in which chitosan and its derivatives have been applied as a sorbent to remove organic

Table 3 Chitosan and its derivatives as sorbents for remediating organic contaminants in wastewater

Chitosan derivatives	Organic contaminants	Reference
Chitosan	POME	Ahmad et al. (2006)
Chitosan	VOME	Geetha Devi et al. (2012)
Chitin (46% DD)	Crude oil	Ummadisingu and Gupta (2012)
Chitosan-alginate micro-shells	DCP and SA	Ding et al. (2009)
Chitosan-montmorillonite nanocomposite	Clopyralid herbicides	Celis et al. (2012)
CuCH nanocomposite	Malathion insecticides (parathion and methyl parathion)	Jaiswal et al. (2012)
D-glucosamine	*p*-benzoquinone	Park et al. (2000)
α-ketoglutaric acid-modified chitosan	BPA	Gong et al. (2010)
Chitosan/γ-Fe_2O_3/fly-ash-cenospheres composites	BPA and TCP	Pan et al. (2011)
Chitosan beads	Quinones	Suzuki et al. (2010)
Chitosan beads	Quinones	Kimura et al. (2012)

Note: *VOME* vegetable oil mill effluent, *POME* palm oil mill effluent, *DD* degree of deacetylation, *CuCH* copper-chitosan, *DCP* 2,4-dichlorophenol, *SA* salicylic acid, *BPA* Bisphenol A, *TCP* 2,4,6-trichlorophenol contaminants from wastewater. Chitosan is known to have a high capacity for sorbing oily contaminants (Yong and Wong 2013). Because chitosan is positively-charged at low pH values, it may be an effective coagulant for anionic organic contaminants such as dyes, tannins and humic acids. Recently, toxic organic chemicals (e.g., phenol and bisphenol A (BPA)) have been remediated by using chitosan incorporated with enzymes (Suzuki et al. 2010), clay (Fan et al. 2007) and Cu(II) hydroxide nanoparticles (Jaiswal et al. 2012).

Oily wastewater (e.g., palm oil mill effluent (POME) and vegetable oil mill effluent (VOME)) causes serious environmental problems. POME is a thick brownish colloidal suspension that contains 95–96% water, 0.6–0.7% oil and grease, 4–5% total solids and pH ranging from 4.0 to 5.0 (Rupani et al. 2010). Chitosan may act as a non-toxic coagulant (Ahmad et al. 2006) and sorbent (Ahmad et al. 2005) of residual oil and suspended solids from POME. Ahmad et al. (2006) found that chitosan performed more efficiently and economically than did other coagulants (e.g., aluminum sulfate (alum) and polyaluminum chloride (PAC)) and sorbents (e.g., bentonite and activated carbon).

Toxic *p*-benzoquinone in treated drinking water is a potential threat to human health (Richardson and Postigo 2012). It is produced from chlorination of acetaminophen in wastewater (Bedner and MacCrehan 2005). Chitosan has been used to detoxify *p*-benzoquinone in aqueous systems (Park et al. 2000). The non-enzymatic reaction between *p*-benzoquinone and D-glucosamine (a monomer of chitosan) occurred at an optimum pH (>7.4) and temperature (35 °C), in which free $–NH_2$ groups in chitosan formed covalent bonds with the *p*-benzoquinone.

Chitosan has been evaluated for remediating anionic organic compounds such as humic substances (Bratskaya et al. 2004) and humate-metal complexes from wastewater (Yan and Bai 2005). Humic substances are widespread natural polymers that (a) adversely affect the aesthetics of waterways; (b) enhance the bioavailability of metal ions by forming metal-humate complexes; and (c) produce carcinogenic by-products with chlorine (e.g., trihalomethanes). Compared to alum, chitosan is more effective and eco-friendly in reducing the chemical oxygen demand (COD) of wastewater that is generated from the food processing industry (Muzzarelli and Muzzarelli 2003). At low pH, the sorption capacity of chitosan for humate decreases by about 25%. The $–NH_3^+$ group in chitin and chitosan is responsible for forming ionic complexes with the negatively charged humate ions (Wan Ngah and Musa 1998). In addition to ionic forces, van der Waals forces contribute to chitosan sorption of tannin and humate ions.

Chitosan composites have been synthesized, with the aim of enhancing its sorption capacity for organic contaminants. Blending chitosan (240 g/kg at pH 4) with montmorillonite increased its CEC and capacity to sorb tannic acid (An and Dultz 2007). Recently, a chitosan-montmorillonite nanocomposite was produced for sorbing anionic herbicide clopyralid from wastewater and soil-water suspension (Celis et al. 2012). High sorption capacity for clopyralid occurred at low pH, because of its abundant $–NH_3^+$ ions in the chitosan-montmorillonite nanocomposite. Desorption of clopyralid increased with higher salt concentrations, indicating a cation exchange sorption mechanism (Celis et al. 2012). Apart from clay, Cu(II) hydroxide has been blended with chitosan to produce CuCH nanocomposites, for remediation of organophosphorous insecticides (i.e., parathion and methyl parathion) in agricultural runoff (Jaiswal et al. 2012). Unlike the chitosan-montmorillonite nanocomposite, sorption of malathion onto a CuCH nanocomposite occurred through complexation. Chitosan-alginate micro-shells have been synthesized by assembling layers of alginate and chitosan, for decontaminating 2,4-dichlorophenol (DCP) and salicylic acid (SA) in wastewater. The chitosan-alginate micro-shells have enhanced sorption capacity, and have a shorter sorption time for DCP and SA, than do poly-styrenesulfonic acid (*PSS*) and poly(allylamine hydrochloride) shells (Ding et al. 2009).

BPA is used to harden polycarbonate plastics and epoxy resins. Its presence in waterways may impact normal functions of endocrine hormone in fishes and humans. Chitosan and its derivatives have been used as a sorbent for BPA and its enzymatic oxidation by-products (i.e., quinones). Chitosan is more effective in the bead form than in solution or in powder form, when used to sorb quinones from treated wastewater (Kimura et al. 2012; Suzuki et al. 2010). Sorption of BPA and trichlorophenol (TCP) from wastewater has been investigated using magnetic chitosan-fly ash composite (Pan et al. 2011). Spent sorbent could be recovered by applying magnetic field to the suspension of treated wastewater. In addition, α-ketoglutaric acid has been blended with chitosan to produce composite resins that are useful for sorbing BPA from wastewater (Gong et al. 2010).

Remediating dyes in textile wastewater is vital because of their adverse effects on human and aquatic life (Chequer et al. 2013). The presence of $–NH_3^+$ groups in chitosan makes it suitable for remediating dye-containing wastewater. The auxochrome groups that consist of acidic (–COOH, –OH and $–SO_3H$) or basic functional groups (–NH–, >N–, $–NH_2$) augment the color intensity of a dye molecule (Nidheesh et al. 2013). The presence of acidic auxochromes increases the negative charge of the dye molecules, enabling ionic interactions with the $–NH_3^+$ groups in chitosan. Chitosan or its derivatives have mainly been applied to remediate acidic or acid-azoic dyes that contain sulfonate groups ($–SO_3^-$) (Table 4). Chitosan has also been used for sorbing congo red (Chatterjee et al. 2007), reactive black 5 (Guibal et al. 2005), reactive red 222 (Wu et al. 2000), direct scarlet B (Annadurai 2000), acid blue 161 (Aksu et al. 2008), acid black 1 and acid violet 5 (Szyguła et al. 2008), acid orange 10, acid red 18, acid red 73 and acid green 25 (Cheung et al. 2007) dyes. In addition, chitosan has been used to immobilize TiO_2 photocatalyst for photo oxidation of dye molecules in wastewater (Zainal et al. 2009).

High molecular weight chitosan may be used as a coagulant to complement the action of conventional aluminum and iron coagulants (Guibal et al. 2006). Dye remediation by chitosan relies on surface charge neutralization of dye molecules. The $–SO_3^-$ groups in the acidic dye are attracted to $–NH_3^+$ groups in chitosan molecule through an electrostatic interaction (Chiou and Li 2003). Moreover, negatively-charged-acid-dyes may be attracted to several $–NH_3^+$ groups in chitosan molecule. The so-called bridging mechanism causes coagulation from the increased molecular weight of dye molecules (Guibal and Roussy 2007).

Table 4 Chitosan and its derivatives as sorbents for remediating dye molecules in wastewater

Dye	Classification/ Polarity	Chitosan derivatives	Reference
Methyl orange	Azoic/Anionic	Chitosan-TiO_2 photocatalyst membrane layers	Zainal et al. (2009)
Metanil yellow (MY)	Azoic/Anionic	TPP and ECH-crosslinked chitosan	Chiou and Chuang (2006)
Congo red (CR)	Diazoic/ Anionic	Chitosan	Chatterjee et al. (2007)
		N, *O*-carboxymethylated chitosan	Wang and Wang (2008)
		Polyalkylmethacrylate-grafted chitosan	Konaganti et al. (2010)
Reactive black 5	Diazoic/ Anionic	Chitosan	Guibal et al. (2005)
		GA and TPP-crosslinked chitosan	Lazaridis and Keenan (2010)
Reactive red 2	Reactive-azoic/ Anionic	TPP and ECH-crosslinked chitosan	Chiou et al. (2004)
Reactive red 222 (RR222)	Reactive-azoic/ Anionic	Chitosan	Wu et al. (2000)
Reactive red 189 (RR189)	Reactive-azoic/ Anionic	ECH-crosslinked chitosan	Chiou and Li (2002)
Reactive blue 2	Reactive/ Anionic	TPP and ECH-crosslinked chitosan	Chiou et al. (2004)
Reactive blue 5 (RB5)	Reactive/ Anionic	MCM-chitosan	Uzun and Güzel (2004)
Reactive blue 15 (RB15)	Reactive/ Anionic	TPP and ECH-crosslinked chitosan	Chiou and Chuang (2006)
Reactive yellow 2	Reactive azoic/ Anionic	TPP and ECH-crosslinked chitosan	Chiou et al. (2004)
Reactive yellow 86	Reactive azoic/ anionic	TPP and ECH-crosslinked chitosan	Chiou et al. (2004)
Direct scarlet B	Direct/Anionic	Chitosan	Annadurai (2000)
Direct red 81	Azoic/Anionic	TPP and ECH-crosslinked chitosan	Chiou et al. (2004)
Acid blue 25	Acidic/Anionic	EDGE-crosslinked chitosan	Kamari et al. (2009)
Acid blue 161 (AB 161)	Acidic/Anionic	Chitosan	Aksu et al. (2008)
Acid black 1	Acidic/Anionic	Chitosan	Szyguła et al. (2008)
Acid violet 5	Acidic/Anionic	Chitosan	Szyguła et al. (2008)

(continued)

Table 4 (continued)

Dye	Classification/ Polarity	Chitosan derivatives	Reference
Acid orange 7 (Orange II)	Azoic/Anionic	TPP and ECH-crosslinked chitosan	Chiou et al. (2004)
		Sulfate-crosslinked chitosan	Xu et al. (2008)
		MCM-chitosan	Uzun and Güzel (2004)
Acid orange 10	Acidic/Anionic	Chitosan	Cheung et al. (2007)
Acid orange 12	Azoic/Anionic	TPP and ECH-crosslinked chitosan	Chiou et al. (2004)
Acid red 14	Azoic/Anionic	TPP and ECH-crosslinked chitosan	Chiou et al. (2004)
Acid red 18	Azoic/Anionic	Chitosan	Cheung et al. (2007)
Acid red 37	Azoic/Anionic	Chitosan	Kamari et al. (2009)
		EDGE-crosslinked chitosan	
Acid red 73	Azoic/Anionic	Chitosan	Cheung et al. (2007)
Acid red 87 (Eosin Y)	Acidic/Anionic	Sulfate-crosslinked chitosan	Chatterjee et al. (2005)
		TPP-crosslinked chitosan nanoparticle	Du et al. (2008)
Acid green 25	Acidic/Anionic	Chitosan	Cheung et al. (2007)
Methylene blue (MB)	Basic/Cationic	Chitosan	Annadurai (2002)
Crystal violet	Basic/Cationic	MCM-chitosan	Uzun and Güzel (2004)

Note: *EDGE* ethylene glycol diglycidyl ether, *TPP* tripolyphosphate, *ECH* epichlorohydrin, *GA* glutaraldehyde, *MCM-chitosan* monocarboxymethylated-chitosan

Attempts have been made to sorb basic dye (methylene blue) by using chitosan (Annadurai 2002). Although increasing sorption temperature and pH augmented the capacity of chitosan to sorb methylene blue (Annadurai 2002), the interaction between the basic dye and chitosan might not be sustained because of their similar functional groups. Chitosan and basic dye have primary (–NH_2) and tertiary amine groups (>N–), respectively. To enhance the sorption of basic dyes, negatively charged functional groups (i.e., –COOH) have been grafted onto –NH_2 and/or –OH groups in chitosan molecules. Grafting of the chitosan molecule with chloroacetic acids has produced monocarboxymethylated chitosan (MCM-chitosan) (Uzun and Güzel 2004), and *N, O*-carboxymethyl-chitosan (*N, O*-CMC) (Wang and Wang 2008). Uzun and Güzel (2004) reported an increase in sorption percentage of MCM-chitosan for acid orange 7 (99%) compared to that of chitosan (12%). Wang and Wang (2008) reported higher sorption capacity of *N, O*-CMC for congo red dye (331 mg/g) than that of chitosan (79 mg/g). The augmented sorption capacity for congo red was attributed to the complexation of dye molecule by –COO^-, –NH_2 and –OH groups in *N, O*-CMC (Wang and Wang 2008).

As previously mentioned, chitosan is structurally unstable under acidic conditions and hence its application as a sorbent is problematic, when remediating dye-containing acidic wastewaters. A crosslinking process is required to prevent the solubilization of chitosan sorbent in acidic dye wastewater (see Chitosan Modification). Permanent crosslinks have been introduced to chitosan using EDGE (Kamari et al. 2009) and ECH (Chiou and Li 2003). Non-permanent, ionic crosslinks may also enhance the stability of chitosan in acid, which is formed when chitosan reacts with divalent anions (sulfate) (Xu et al. 2008) or polyvalent anions (citrate) (Trung et al. 2003). Addition of ionic crosslinks also increased the amorphicity of chitosan, thereby contributing to higher sorption capacity for anionic dyes.

Spent chitosan sorbent is regenerated by treatment with alkaline solutions. A decrease in the sorption capacity of chitosan for dye occurs as pH increases. About 20% of dye molecules were desorbed from the chitosan beads when the solution pH was increased to 12 (Chatterjee et al. 2007). Chitosan crosslinked with ECH had greater sorption capacity than GA- and EDGE-crosslinks (Chiou et al. 2003; Chiou and Li 2003). Crosslinked chitosans have been studied for sorption of metanil yellow, reactive blue 15 (Chiou and Chuang 2006); reactive red 2, acid red 14, reactive yellow 2, reactive yellow 86, reactive blue 2, direct red 81, acid orange 7, acid orange 12 (Chiou et al. 2004); reactive black 5 (Guibal et al. 2006); acid blue 25, acid red 37 (Kamari et al. 2009); acid red 18, acid red 73 (Cheung et al. 2007) and acid red 87 (Chatterjee et al. 2005; Du et al. 2008). Crosslinked chitosan may be used in the permeable reactive barriers (PRBs) for remediating dyes in soil (Lazaridis and Keenan 2010). Chitosan beads crosslinked with GA and inoculated with TPP have a greater affinity and retardation factor for reactive black 5 as compared to soil.

Although crosslinking is likely to decrease the content of $-NH_3^+$ groups in chitosan's structure, its sorption capacity for dye may not be decreased, because crosslinked chitosan has an augmented dye sorption capacity, as compared to non-crosslinked chitosan (Chiou et al. 2004). Parameters such as low pH may enhance sorption capacity for dye molecules. Certain interactions between chitosan and dye molecules may also affect dye sorption capacity of chitosan. For instance, dyes interact with high molecular weight chitosan via coagulation mechanisms involving charge neutralization and bridging effects (Szyguła et al. 2008). Remediation of large, planar direct dye molecules may involve van der Waals forces (Mazengarb and Roberts 2009). Sorption of reactive dyes may occur with the C_6-OH group in chitosan (Shukla and Pai 2005). The stability of hydrogen bonds between the dye and the chitosan molecule also depends on the polarity of $-NH_2$ groups, which may be influenced by its spatial arrangement (e.g., $-NH_2$, $>N-$, $-N=$) (Ikeda et al. 2008). Sorption degree of dyes is affected by surface and pore diffusion processes in the porous chitosan (Cheung et al. 2007). Dye molecules may form aggregates, even at low dye concentrations, thereby, decreasing sorption capacity of porous chitosan (Walker and Weatherley 2001).

Chitosan and its derivatives have an indirect role in bioremediation of organic contaminants in soil and aqueous systems. Chitosan may be used as (a) an

Table 5 Chitosan and its derivatives as immobilizing agents for remediating wastewater and soil with organic contaminants

Chitosan	Immobilized entities	Organic contaminants	Reference
Chitosan	*Rhodococcus corynebacterioides*	Crude oil	Gentili et al. (2006)
Chitosan	*Sphingobium* sp. *P2*	Automotive lubricant	Khondee et al. (2012)
Chitosan beads	TYR	*p*-Cresol	Yamada et al. (2005)
		4-Ethylphenol	
		4-*n*-propylphenol	
		4-*n*-butylphenol	
		p-Chlorophenol	
		4-Tert-butylphenol	
Chitosan membrane	Ni/Fe and Pd/Fe nanoparticles	TCE and DCB	Tee and Bhattacharyya (2008)
Chitosan-polyvinyl alcohol membrane	Laccase	DCP	Xu et al. (2013)
GA-crosslinked chitosan	TYR	Phenol	Shao et al. (2007)
Chitosan-SiO_2 gel matrix			
Chitosan-coated alginate-polyvinyl alcohol microspheres	*Mycobacterium frederiksbergense*	Pyrene	Sarma et al. (2011)
Chitosan-carrageenan gels	*Pseudomonas putida* (NICM 2174)	Chlorophenol	Jianlong and Yi (1999)
Chitosan-alginate composite	*Pseudomonas*, *Bacillus*, *Zoogloea* and *Micrococcus*	Unspecified pesticides (in soil)	Si et al. (2010) (2009, patent appl date)

Note: *TYR* tyrosinase, *TCE* trichloroethylene, *DCB* 2, 2′-dichlorobiphenyl, *DCP* 2,4-dichlorophenol, *GA* glutaraldehyde

immobilizing material for microbes or enzymes, or (b) a solubilizing agent of organic contaminants. Crosslinked chitosan can also be used as a sole immobilizing material. Other materials have been blended with chitosan to form composites for immobilizing reactive microbes or enzymes (Table 5). For example, fungal tyrosinase enzyme (TYR) has been immobilized using chitosan (Yamada et al. 2005) and chitosan-SiO_2 gel (Shao et al. 2007) to remediate phenol compounds in wastewater. TYR catalyzes the oxidation of phenol to *o*-quinones, which are then adsorbed by chitosan (Ikehata and Nicell 2008). The sorption of *o*-quinones by chitosan is important if inactivation of TYR is to be avoided. When avoided, TYR can be reused for further remediation of wastewater. Other polymeric materials such as polyvinyl alcohol have also been used together with chitosan in the immobilization of the laccase enzyme to remediate 2,4-dichlorophenol (DCP) (Xu et al. 2013).

Chitosan has been used to immobilize reactive microbes, such as *Sphingobium* sp. *P2* (Khondee et al. 2012) and *Rhodococcus corynebacterioides* (Gentili et al. 2006), for degrading lubricants and crude oils in water, respectively. Another bacteria, *Mycobacterium frederiksbergense* was immobilized in chitosan, and was coated onto alginate-polyvinyl alcohol beads for degrading pyrene in silicone oil (Sarma et al. 2011). Furthermore, chitosan may be blended with negatively charged biopolymers, such as carrageenan for immobilizing *Pseudomonas putida* (NICM 2174) (Jianlong and Yi 1999), alginate for immobilizing a variety of microbes (i.e., *Pseudomonas, Bacillus, Zoogloea, Micrococcus*, etc.) (Si et al. 2010) (2009, patent appl date), and carboxymethylated biopolymers (i.e., carboxymethyl cellulose and carboxymethyl chitosan) for immobilizing phosphate-solubilizing bacteria (i.e., *Bacillus, Pseudomonas*, *Rhizobium* or *Agrobacterium*), *Azospirillum brasilense* and *Alcaligenes faecalis* (Zhang et al. 2011) (2009, patent appl date).

Chitosan is also useful for remediating oil-contaminated soils or seawater by facilitating microbial degradation of oil contaminants. Oily, non-water-soluble contaminants are sorbed and concentrated in chitosan, improving interaction with microbes for effective degradation. Addition of chitin and chitosan significantly reduced degradation time for heavy oils in seawater by *Pseudomonas* sp. (Setti et al. 1999). The efficiency of chitin and chitosan in the remediation of oil-contaminated beach sediment was compared with a slow-releasing fertilizer (Osmocote). Even though chitosan alone has higher oil sorption capacity (2.2 g/g) than chitin (0.24 g/g), it requires a nutrient supply to induce biodegradation by microbes. Addition of chitosan has increased biodegradation of polyaromatic hydrocarbon (PAH) compounds, possibly from higher availability of PAH to the microbial biomass (Xu et al. 2005).

3.4 Chitosan-Based Sensors

A sensor is a device that provides analytical information about an analyte (e.g., identifying or characterizing the analyte and its concentration) by detecting signals generated from a chemical and/or a biological reaction (Hulanicki et al. 1991). These signals are usually measured by using optical (e.g., absorbance, reflectance or fluorescence spectrophotometry) (Fan et al. 2008), or electrochemical detectors (amperometry, voltametry, conductimetry and potentiometry) (Hanrahan et al. 2004). An amperometric sensor is capable of rapid, real-time detection of contaminants, as compared to colorimetric and spectrophotometric-based sensors (Brett 2001). Incorporating sensing elements in the amperometric sensor enhances detection sensitivity and selectivity for analytes, with greater reproducibility of results (Du et al. 2007c).

Chitosan and its derivatives are mostly biocompatible, making them suitable for immobilizing sensing elements, such as enzymes (e.g., TYR, acetylcholinesterase

(AChE), horseradish peroxidise (HRP), organophosphorus hydrolase (OPH), and alkaline phosphatase (ALP)), and nanoparticles (e.g., gold and Fe_3O_4). Immobization of sensing elements may include a crosslinking process to (a) prevent the loss of incorporated sensing elements, and (b) stabilize metallic/ magnetic materials in the sensors (Radhakumary and Sreenivasan 2012; Sugunan et al. 2005) (Table 6). Although GA has been used in crosslinking processes, it is toxic to all living organisms and may decrease the sensitivity of sensors by denaturing the immobilized enzyme (Leung 2001). Non-toxic chemicals have been used to replace GA in the crosslinking process. These chemicals include organic polyprotic anions (e.g., alginate and carageenan) or clays (e.g., layered double hydroxide and laponite) (Fan et al. 2007). Co-immobilization of chitosan and polyprotic anion improved the ionic interactions of $–NH_3^+$ groups and negative surfaces of polyanions, thereby, improving the mechanical strength of the host matrix for enzymes (Han et al. 2007). Chitosan is also used as a ‘capping’ agent to stabilize and reduce gold nanoparticles in chemosensors (Radhakumary and Sreenivasan 2012; Sugunan et al. 2005).

Chitosan has been used as a sensing element in chemosensors for detecting metals (Table 6). The $–NH_2$ and –OH groups in the chitosan molecule form complexes with metal-based analytes (Wu et al. 2010). The affinities of metal ions to chitosan are different, depending on the presence of soft, borderline, or hard Lewis bases of pristine or modified chitosan. Thin- film chitosan composites have been modified with polyallylamine, increasing the $–NH_2$ group content in thin film for qualitative detection of metal ions in wastewater (Schauer et al. 2004). Cathell et al. (2008) produced a colorimetric sensor containing thiol groups to chitosan film to enhance the selectivity for Hg(II) ions. Complexation of Hg(II) ions by thiolated groups changes the thickness, refractive index and color of the chitosan film. These optical changes were measured to identify and quantify Hg(II) ions in wastewater (Cathell et al. 2008). The thiolated sensor film may detect other metal ions (in the d-block of the periodic table) that form color complexes with the thiol groups.

Surface plasmon resonance (SPR) is a method commonly applied in colorimetric sensors to measure the monolayer thicknesses of adsorbed contaminant molecules on a conducting metal surface (typically gold or silver) (Eustis and El-Sayed 2006). The principle of SPR is based on the oscillation of charge density arising from the interaction of incidental light with the metal surface. Chitosan-based SPR sensor consists of a thin layer of chitosan, coated onto the adjacent metal surface. Sorption of metal ions by chitosan changes the refractive index of the coated metal surface, and is measured to determine the concentration of metal ions (Yu et al. 2004). Chitosan has been used in the SPR sensor to enhance sensitivity for Hg(II) and Pb (II) (Abdi et al. 2011); Fe(III) (McIlwee et al. 2008); Cu(II), Zn(II) and Mn(II) (Fen et al. 2011); and Cu(II) ions (Lin et al. 2012). However, chitosan is not suitable for detecting other metal(loid) complexes (e.g., Al(III), As(III) and As(VI), etc.) due to the colorless nature of most of the metal(loid)-amine complexes. To overcome this problem, a fluorescent dye is grafted onto the sensor surface to augment signals of the colorimetric detectors. Rhodamine (Meng et al. 2012) and dansyl chloride

Table 6 Chitosan and its derivatives in bio- and -chemosensors

Chitosan derivatives	Immobilized enzymes or nanoparticles	Analyte	Reference
GA-crosslinked chitosan	Tyrosinase-labeled, nano-gold colloids	Catechol, *p*-cresol, phenol	Liu et al. (2004)
Chitosan-nano ZnO composite	TYR		Li et al. (2006)
Chitosan-nano Fe_3O_4 nanocomposite	TYR		Wang et al. (2008)
Chitosan	TYR	Phenol	Abdullah et al. (2006)
Ru(byp)$_2$Cl$_2$-crosslinked chitosan	TYR	Phenol	Zhang and Ji (2010)
Chitosan-laponite composite	PPO	Catechol, *p*-cresol, phenol, and *m*-cresol	Fan et al. (2007)
Chitosan-LDH composite	PPO		Han et al. (2007)
Electropolymerized chitosan	AChE	Carbaryl (insecticide)	Du et al. (2007a)
GA-crosslinked chitosan-MWNT composite	AChE	Triazophos (insecticide)	Du et al. (2007b)
GA-crosslinked chitosan-MWNT composite	AChE	Carbaryl (insecticide)	Du et al. (2007c)
Chitosan-sol gel composite	ALP	2,4-D and 2,4,5-T (herbicides), Carbofuran and *α*-endosulfan (insecticides), and Hg(II), Cd(II), Cu(II), Zn(II), Ag(I)	Loh et al. (2008b)
Chitosan-sol gel-nano Fe_3O_4 composite	ALP	2,4-D (herbicide)	Loh et al. (2008a)
Chitosan	HRP	Picloram (herbicide)	Tang et al. (2008)
Chitosan	HRP	Cyanide	Wang et al. (2010b)
Chitosan	Gold nanoparticles	Zn(II) and Cu(II)	Sugunan et al. (2005)
Chitosan	Gold nanoparticles	Cyanide	Radhakumary and Sreenivasan (2012)
Chitosan-PTAA composite	OPH	Paraoxon (insecticide metabolite)	Constantine et al. (2003)
Chitosan		Catechol	Dykstra et al. (2009)

(continued)

Table 6 (continued)

Chitosan derivatives	Immobilized enzymes or nanoparticles	Analyte	Reference
HDACS-crosslinked chitosan		Fe(III)	McIlwee et al. (2008)
		Fe(III) and Cu(II)	Praig et al. (2009)
		Cr(VI)	Fahnestock et al. (2009)
Electropolymerized chitosan-polypyrole composite		Hg(II) and Pb(II)	Abdi et al. (2011)
Electropolymerized GA-crosslinked-chitosan		Cu(II), Zn(II) and Mn (II)	Fen et al. (2011)
Chitosan		Cu(II)	Lin et al. (2012)
Thiolated chitosan-MWNT nanocomposite		Hg(II)	Deng et al. (2010)
Dansylamide-grafted chitosan nanoparticle		Hg(II)	Wang et al. (2012)
Rhodamine-grafted chitosan		Hg(II)	Meng et al. (2012)
HDACS-crosslinked chitosan		Various metal ions	Schauer et al. (2004)
L-cysteine, thioglycolic acid, and 2-iminothiolane grafted chitosan		Various metal ions	Cathell et al. (2008)

Note: *TYR* tyrosinase, *PPO* polyphenol peroxidise, *AChE* acetylcholinesterase, *ALP* alkaline phosphatase, *GA* glutaraldehyde, *HRP* horseradish peroxidise, *LDH* layered double hydroxides, *MWNT* multiwall carbon nanotube, *OPH* organophosphorous hydrolase, *2,4-D* 2,4-dichlorophenoxyacetic acid, *2,4,5-T* 2,4,5-trichlorophenoxyacetic acid, *PTAA* polythiophene-3-acetic acid, *HDACS* hexamethylene-1,6-diaminocarboxysulfonate

(Wang et al. 2012) were grafted onto chitosan films to enhance detection of Hg (II) ions. Alternatively, introduction of a thiol group could increase chemisorption of Hg(II) ions to a chitosan sensor, where signal was measured using anodic stripping voltametry (Deng et al. 2010).

A chitosan film has been studied for detecting low level catechol in wastewater (1 mM); the film has a detection limit of about 0.2 mM for a 10 min reaction time (Dykstra et al. 2009). Although chitosan has not yet been applied successfully in a commercial setting for organic contaminant sensing, it has great potential as a molecular-imprinted sensor for detecting specific contaminants. This initial success may pave the way for developing a cheap, enzyme-free sensor that is commercially cost effective.

4 Conclusions and Future Research

We have demonstrated in this review that chitosan and its derivatives show promise for various environmental applications. Such applications range from remediation of organic and inorganic contaminants to development of sensors for soil and water contaminates. The prospects for commercial use of chitosan and its derivatives have grown significantly as their versatility and biodegradability have been enhanced. However, chitosan's cost effectiveness in comparison to other biomaterials is questionable because processing it for use requires substantial amounts of high-grade alkali. Unless economical and reliable processing and production methods are developed, use of chitosan for environmental applications will remain commercially unviable. Enzymatic or microbial methods for chitosan processing appear to be economic; however, further work is required to demonstrate their commercial economic viability.

Alternative raw materials such as fungi and insects have been used for chitosan production to overcome seasonal availability of the major source (i.e., seafood-derived chitins). Fungal mycelium is a significant chitin source due to the growing consumption of mushrooms worldwide. The mycelium-containing spent mushroom waste may be enzymatically processed to produce more affordable, low-grade chitosan for large-scale environment applications (Tsigos et al. 2000). However, more research is needed to determine the feasibility of using low-grade fungi-derived chitosan for environmental applications.

Application of chitosan in acidic environments has been challenged by its high solubility, and low structural stability. This limitation can be overcome by modifying chitosan's structure via crosslinking. Such modifications not only improve the stability of chitosan, but also enhances its physical characteristics, such as surface area, porosity, hydraulic conductivity and permeability, etc.. Because chitosan predominantly contains positively charged groups at low pH, certain crosslinks also introduce negative charges that enhance chitosan's sorption capacity for metal cations. However, the sorption capacity of modified chitosan varies with the type of crosslink and methodologies used for crosslinking. Further research is required to ascertain what types of crosslinking agents best enhance sorption capacities for various cations.

Chitosan has been used to remediate a vast array of contaminants. Heavy metals, for example, have been remediated by using pure chitosan and modified chitosans, and by combining chitosan with microorganisms. Low molecular weight chitosan derivatives have been effective in enhancing phytoremediation of certain heavy metals. However, the mechanism by which plant uptake of metals is enhanced in the presence chitosan is not clear, and must be clarified by performing additional research. Similarly, chitosan and its derivatives have been effective for remediating anionic organic contaminants such as dyes, tannins and humic acids, due to the presence of having positive surface charges at low pH. Other organic contaminants, such as phenol, bisphenol-A, PCBs, pharmaceuticals, *p*-benzoquinone, oil-based wastewater, and herbicides have been remediated by using chitosan. Chitosan has the potential to remediate many other organic contaminants, and discovering such contaminants will be an interesting topic for further research.

Organo-clays have been effective in remediating both organic and inorganic contaminants. Chitosan has been blended with some clay minerals such as montmorillonite to enhance the surface area for sorbing both organic and inorganic contaminants. However, limited research has been conducted in this area, and a great potential exists for synthesis of sorbents made up of different organo-clays and chitosan.

Recently, incorporation of chitosan in nano zero valent iron (nZVI) particles has been attempted to form chitosan nano-composites, which have subsequently been used to oxidize certain organic contaminants. However, the production cost of such nano-composites is high. Hence, these composits must be reusable, which has been successfully achieved by incorporating magnetic nanoparticles. As nZVI is increasingly being used in contaminant remediation activities, more research is required to study the suitability of chitosan for minimizing aggregation and oxidation of these nanoparticles. Moreover, little is known about the ecological and human health effects of these new nanoparticles, so the need to acquire safety data must also be met.

Chitosan has been suitable for use in developing sensors for many metals (e.g., Cu, Fe, Hg, Mn, Pb and Zn), organic compounds (e.g., enzymes) and nanoparticles (e.g., Au and Fe) by using immobilization and crosslinking processes. Chitosan could not be successfully applied as a sensor to detect metalloids such as Al(III), As (III) and As(VI), etc. because the metalloid-amine complex is colorless. Certain fluorescent dyes can be added to overcome this issue. For example, detection of Hg was achieved by using rhodamine and dansyl chloride. However, more research is required to investigate the potential of chitosan in sensing these and other metal (loid)s. Although chitosan has not yet been applied successfully for organic contaminant sensing, it is known to have the potential as a molecular-imprinted sensor for detecting specific contaminants.

5 Summary

Chitosan originates from the seafood processing industry and is one of the most abundant of bio-waste materials. Chitosan is a by-product of the alkaline deacetylation process of chitin. Chemically, chitosan is a polysaccharide that is soluble in acidic solution and precipitates at higher pHs. It has great potential for certain environmental applications, such as remediation of organic and inorganic contaminants, including toxic metals and dyes in soil, sediment and water, and development of contaminant sensors.

Traditionally, seafood waste has been the primary source of chitin. More recently, alternative sources have emerged such as fungal mycelium, mushroom and krill wastes, and these new sources of chitin and chitosan may overcome seasonal supply limitations that have existed. The production of chitosan from the above-mentioned waste streams not only reduces waste volume, but alleviates pressure on landfills to which the waste would otherwise go.

Chitosan production involves four major steps, viz., deproteination, demineralization, bleaching and deacetylation. These four processes require excessive usage of strong alkali at different stages, and drives chitosan's production cost up, potentially making the application of high-grade chitosan for commercial remediation untenable. Alternate chitosan processing techniques, such as microbial or enzymatic processes, may become more cost-effective due to lower energy consumption and waste generation.

Chitosan has proved to be versatile for so many environmental applications, because it possesses certain key functional groups, including –OH and $–NH_2$. However, the efficacy of chitosan is diminished at low pH because of its increased solubility and instability. These deficiencies can be overcome by modifying chitosan's structure via crosslinking. Such modification not only enhances the structural stability of chitosan under low pH conditions, but also improves its physicochemical characteristics, such as porosity, hydraulic conductivity, permeability, surface area and sorption capacity.

Crosslinked chitosan is an excellent sorbent for trace metals especially because of the high flexibility of its structural stability. Sorption of trace metals by chitosan is selective and independent of the size and hardness of metal ions, or the physical form of chitosan (e.g., film, powder and solution). Both –OH and $–NH_2$ groups in chitosan provide vital binding sites for complexing metal cations. At low pH, $–NH_3^+$ groups attract and coagulate negatively charged contaminants such as metal oxyanions, humic acids and dye molecules.

Grafting certain functional molecules into the chitin structure improves sorption capacity and selectivity for remediating specific metal ions. For example, introducing sulfur and nitrogen donor ligands to chitosan alters the sorption preference for metals.

Low molecular weight chitosan derivatives have been used to remediate metal contaminated soil and sediments. They have also been applied in permeable reactive barriers to remediate metals in soil and groundwater. Both chitosan and modified chitosan have been used to phytoremediate metals; however, the mechanisms by which they assist in mobilizing metals are not yet well understood. In addition, microbes have been used in combination with chitosan to remediate metals (e.g., Cu and Zn) in contaminated soils.

Chitosan has also been used to remediate organic contaminants, such as oil-based wastewater, dyes, tannins, humic acids, phenols, bisphenol-A, *p*-benzoquinone, organo-phosphorus insecticides, among others.

Chitosan has also been utilized to develop optical and electrochemical sensors for in-situ detection of trace contaminants. In sensor technology, naturally-derived chitosan is used primarily as an immobilizing agent that results from its enzyme compatibility, and stabilizing effect on nanoparticles. Contaminant-sensing agents, such as enzymes, microbes and nanoparticles, have been homogeneously immobilized in chitosan gels by using coagulating (e.g., alginate, phosphate) or crosslinking agents (e.g., GA, ECH). Such immobilization maintains the stability of sensing elements in the chitosan gel phase, and prevents inactivation and loss of the sensing agent.

In this review, we have shown that chitosan, an efficient by-product of a waste biomaterial, has great potential for many environmental applications. With certain limitations, chitosan and its derivatives can be used for remediating contaminated soil and wastewater. Notwithstanding, further research is needed to enhance the physicochemical properties of chitosan and mitigate its deficiencies.

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Mechanisms of Hexavalent Chromium Resistance and Removal by Microorganisms

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Contents

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1 Introduction

Chromium is a naturally occurring element found in rocks, animals, plants, soil and in volcanic dust and gases. It exists in different oxidation states that range from +2 to +6. The most stable forms are Cr(VI) and Cr(III), although they significantly differ in biological, geochemical and toxicological properties. Cr(III) occurs naturally in the environment at a narrow concentration range and is considered to be less toxic than Cr(VI). Hexavalent chromium is used extensively in industrial processes such as electroplating, tanning, textile dyeing, corrosion inhibition and wood treatment, all of which produce discharge of chromium-containing effluents (Lauwerys et al. 2007). The high solubility of Cr(VI) makes it a hazardous contaminant of water and soil when discharged by industries that produce or utilize chromium. When it is released to the environment, Cr(VI) is a potential contaminant of groundwater that can participate in trophic transfer in food chains. The United States Environmental Protection Agency has identified Cr(VI) as one of the 17 chemicals posing the greatest threat to humans (Marsh and McInerney 2001). The permissible limit for total chromium in drinking water is 0.05 mg/L (WHO 2004).

The origin of this paper was our belief that an improved understanding of how microbes resist Cr(VI) can serve to provide insight into strategies for removing it from the environment. Therefore, in this paper, we attempt to describe the literature that addresses the biological remediation of Cr(VI) by various microorganisms such as bacteria, yeasts, fungi and algae. We have also included selected genetically engineered microorganisms that have shown adaptability to Cr(VI) exposure by either acquiring resistance to Cr(VI) toxicity or by participating in detoxification processes to advance their own survival through bioconversion of toxic Cr(VI) to relatively less toxic Cr(III).

2 Toxicity of Chromium

The range of chromium toxicity for most agronomic plants varies from 5 to 100 mg/kg of available chromium in soil (Ghosh and Singh 2005). Because of its high oxidizing potential, Cr(VI) causes mutagenic and carcinogenic effects on biological organisms. Cr(VI) does not interact directly with DNA, hence its genotoxicity is attributed to its intracellular reduction to Cr(III) via reactive intermediates. The resulting types of DNA damage that are produced can be grouped into two categories: (1) oxidative DNA damage and (2) Cr(III)-DNA interactions (Sobol and Schiestl 2012).

Because of its structural similarity to sulfate (SO_4^{2-}), CrO_4^{2-} crosses the cell membrane in some species via the sulfate transport system (Ksheminska et al. 2005). Under normal physiological conditions, after crossing the membrane Cr(VI) reacts spontaneously with intracellular reductants (e.g., ascorbate and glutathione) to generate the short-lived intermediates Cr(V) and/or Cr(IV), free radicals and the end-product Cr(III). Cr(V) undergoes a one-electron redox cycle to regenerate Cr(VI) by transferring the electron oxygen. The process produces reactive oxygen species (ROS), including single oxygen (O) and superoxide (O^{2-}) (Cheng et al. 2009), hydroxyl (OH) and hydrogen peroxide (H_2O_2) radicals (McNeill and McLean 2012) that easily combine with DNA-protein complexes. Therefore, Cr(IV) binds to cellular materials and deters their normal physiological functions (Cervantes et al. 2001). The genotoxic effects of the Cr ion however cannot be solely explained by the action of ROS. Intracellular cationic Cr(III) complexes also interact electrostatically with negatively charged phosphate groups of DNA, which could affect replication, transcription and cause mutagenesis (Cervantes et al. 2001). Moreover, Cr(III) interferes with DNA replication to produce an increased rate of transcription errors in the cell's DNA. Additionally, Cr(III) may alter the structure and activity of enzymes by reacting with their carboxyl and thiol groups (Cervantes et al. 2001).

Occupational exposure to chromium was identified as an important risk factor for lung cancer. This metal also irritates airways, causes nasal and skin ulcerations and lesions, causes perforation of the nasal septum, asthma, dermatitis and other allergic reactions (Halasova et al. 2009). Ingesting Cr(VI) causes stomach and intestinal damage that may lead to cancer. In lab animals, Cr(VI) damages sperm and male reproductive systems (Kim et al. 2012), and in some cases, has damaged the developing fetus (Asmatullah and Shakoori 1998).

3 Microorganisms Implicated in Cr(VI) Detoxification

A variety of microorganisms have been identified as having the capacity to remove Cr(VI) contamination. The microbes that retain such properties have been isolated from a diverse range of environments, both those contaminated and uncontaminated with Cr(VI). Below, we describe the classes of microbes that have displayed potential for reducing or removing Cr contamination.

3.1 Bacteria

Microbial Cr(VI) reduction was first reported in the late 1970s, when Romanenko and Koren'Kov (1977) observed a Cr(VI) reduction capability in *Pseudomonas* species grown under anaerobic conditions. The active bacterial strain, isolated from sewage sludge, was classified as *Pseudomonas dechromaticans*. Since then, several researchers have isolated several microorganisms that catalyze the reduction of Cr (VI) to Cr(III) under varying conditions.

Initially, interest was focused on facultative anaerobic bacteria such as *Aerococcus, Micrococcus* and *Aeromonas* (Srinath et al. 2001), followed by bacteria capable of reducing Cr(VI) aerobically like *Thermus scotoductus* (Opperman and van Heerden 2008) and anaerobically such us *Achromobacter* sp. (Zhu et al. 2008). As will be explained below, the mechanisms of Cr (VI) reduction depend strongly on the oxygen requirements of the bacterium in question. Actinomycetes have also been reported to reduce Cr(VI). Polti et al. (2007) identified 11 Cr(VI) resistant strains, ten from the genus *Streptomyces* and one from *Amycolatopsis*. Recently, Sugiyama et al. (2012) isolated *Flexivirga alba* with Cr(VI) reducing activity that is stimulated by molasses.

Bacteria endowed with the capacity to reduce Cr(VI) levels are named chromium-reducing bacteria (CRB) (Somasundaram et al. 2009). CRB are generally isolated from industrial effluents, especially those from tanneries (Farag and Zaki 2010; Chandhuru et al. 2012), and textile (Çetin et al. 2008) and electroplating manufacturing (Seema et al. 2012). CRB are also isolated from soil contaminated with these effluents (Sayel et al. 2012; Sharma and Adholeya 2012).

Monocultures of different bacterial strains have been used in most Cr (VI) bioremediation studies (Zahoor and Rehman 2009; He et al. 2011; Farag and Zaki 2010). However, in nature, single species seldom survive in a complex environment. Therefore, using pure cultures under controlled lab conditions may not emulate actual environmental conditions, particularly in highly contaminated areas that have more than a single metal present. According to Sannasi et al. (2006), bacteria are more stable and survive better when they exist in mixed culture. In addition, consortia of cultures are metabolically superior for removing metals and are more suitable for field application, because the organisms are more competitive and are more likely to survive (Kader et al. 2007). Considering these advantages, other researchers have found that consortia cultures isolated from the environment offer more efficient Cr(VI) reduction (Chen and Gu 2005; Piñón-Castillo et al. 2010; Tahri Joutey et al. 2011).

Biological treatment of Cr(VI)-contaminated wastewater may be difficult because the metal's toxicity can kill the bacteria. Accordingly, to protect the cells, cell immobilization techniques have been employed by several researchers (Elangovan et al. 2010; Pang et al. 2011; Murugavelh and Mohanty 2013a), because (1) the biofilm-bound cells can tolerate higher concentrations of Cr(VI) than planktonic cells, and (2) they allow easy separation of the treated liquid from the biomass (Harrison et al. 2007).

Considering the deleterious impact of certain physicochemical methods and need to identify alternative technologies for reducing/destroying chromium toxicity, researchers have recently focused on abatement of Cr(VI) toxicity by using plant-growth-promoting rhizobacteria (PGPR) (Chaturvedi 2011).

PGPR are naturally occurring soil bacteria that aggressively colonize plant roots and benefit plants by providing growth promotion (Saharan and Nehra 2011). The use of soil bacteria (often PGPB) as adjuncts in metal phytoremediation can significantly facilitate the growth of plants in the presence of high (and otherwise inhibitory) metal levels (Glick 2010). To increase the efficiency of contaminant extraction, applying plants along with selected microorganisms may be beneficial; such a technique is called rhizoremediation (Jing et al. 2007). Among bacterial isolates, PGPR like *P. putida* P18 and *P. aeruginosa* P16 (Dogan et al. 2011), *P. corrugate* 28 (Christl et al. 2012), *Bacillus* sp. PSB10 (Wani and Khan 2010) are reported to be capable of restoring chromium contaminated sites. Tiwari et al. (2013) observed that when a consortium of *Bacillus endophyticus*, *Paenibacillus macerans*, and *Bacillus pumilus* was inoculated in the rhizospheric zone of *S. munja*, it not only enhanced metal uptake through mobilization, but also promoted plant growth. In addition, bacterial action may change metal speciation to make metals more water soluble and, therefore, more amenable to plant uptake (Tiwari et al. 2013).

3.2 Yeasts

The principal reason that yeasts are resistant to chromium relates more to their limited ion uptake rather than to biological reduction of Cr(VI) to Cr(III); such decreased uptake means decreased absorption (Raspor et al. 2000) and bioaccumulation in yeast cells (Ksheminska et al. 2005). In chromate-resistant strains of *Candida maltosa*, NAD-dependent chromate-reducing activity was discovered to take place mainly in the soluble protein fraction, with the membrane fraction being less active (Ramírez-Ramírez et al. 2004). Recently, it has been discovered that Cr(VI) detoxification occurs via extracellular reducing substances that are secreted by the yeast cells (Ksheminska et al. 2006) such as sulfate and riboflavin (Fedorovych et al. 2009). Indeed, many yeast strains are known to biotransform Cr(VI) to the less toxic Cr(III); examples include *S. cerevisiae*, *Rhodotorula pilimanae*, *Yarrowiali polytica* and *Hansenula polymorpha* (Ksheminska et al. 2006), *Pichia guilliermondii* (Ksheminska et al. 2008) and *Rhodotorula mucilaginosa* (Chatterjee et al. 2012).

Bahafid et al. (2011) found that Cr(VI) removal by *P. anomala* initially involves adsorption on functional groups (e.g., carboxyl group, amide I, amide II, amide III, polysaccharides and sulfonate) of cell surfaces, followed by intracellular accumulation and reduction of Cr(VI) to Cr(III). Bahafid et al. (2013) also discovered that three yeasts (viz., *Cyberlindnera fabianii, Wickerhamomyces anomalus* and *Candida tropicalis*) could be used to effectively remove Cr(VI) via adsorption from contaminated sites.

Ksheminska et al. (2008) suggested that Cr(VI) gains entrance into yeast cells in an oxy-anionic form in bacteria, i.e., via sulfate-specific transport systems. The genes involved in sulfate and chromate transport have been identified. Microbial cells are often impermeable to Cr(III), possibly because they form complexes that have low solubility. The mechanism of such transport is unknown, and it is unclear if the known metal transport systems are responsible for the accumulation of Cr(III) in the cells. It is also unclear as to whether there is a specific system to transport this cation in yeasts.

Ksheminska et al. (2008) identified yeasts as convenient organisms to study bioremediation, because some strains are capable of growing in matrices that have high concentrations of chromium compounds and adsorb or accumulate significant quantities into cells and transform them via chelation to less toxic forms.

3.3 Fungi

Most studies on fungi have claimed that Cr(VI) was removed from aqueous solution through an "adsorption mechanism", and that anionic chromate ions bind to positively charged groups (e.g., amines) of the dead fungal biomass. The chromium binding sites on fungal cell surfaces were most likely carboxyl and amine groups for *Trichoderma* species (Padma and Bajpai 2008). Park et al. (2005a) reported that *A. niger* could reduce Cr(VI) to Cr(III) through a redox reaction unrelated to any enzyme activity. They also found that the dead fungal biomass of four fungal strains (viz., *Aspergillus niger*, *Rhizopus oryzae*, *Saccharomyces cerevisiae* and *Penicillium chrysogenum*) may be used to convert toxic Cr(VI) into the less toxic or nontoxic Cr(III) form (Park et al. 2005b). Therefore, Cr(VI) can be removed from aqueous solution by employing nonliving biomass through two mechanisms: (1) direct reduction: Cr(VI) is directly reduced to Cr(III) in the aqueous phase by contact with the electron-donor groups of the biomass, i.e., groups having lower reduction potential values than that of Cr(VI) (+1.3 V). (2) indirect reduction, which consists of three steps: (a) binding of Cr(VI) anionic species to the positively charged complexing groups present on the biomass surface; (b) reduction of Cr (VI) to Cr(III) by adjacent electron-donor groups and (c) release of the Cr(III) ions into the aqueous phase from electronic repulsion between the positively charged groups and the Cr(III) ions, or complexation of Cr(III) with adjacent groups capable of Cr-binding. This discovery led Park et al. (2006) to conclude that the mechanism of Cr(VI) removal by biomaterials is not "anionic adsorption". Rather, it is an "adsorption coupled reduction".

Other fungal species are also able to reduce Cr(VI) to Cr(III). Examples are: *Hypocrea tawa* (Morales-Barrera et al. 2008) and *Paecilomyces lilacinus* (Sharma and Adholeya 2011). Das and Guha (2009) reported that reduction of chromate ions takes place by chromate reductase activity of cell-free extracts of *Termitomyces clypeatus*. In contrast, the mechanisms of Cr(VI) reduction in *Aspergillus* sp. N2 and *Penicillium* sp. N3 were enzymatically reduced and sorbed to mycelia (Fukuda et al. 2008).

Results of Fourier transform infrared spectroscopy analysis of *Coriolus versicolor* suggested that amino, carboxylate and thiol groups from fungal cell walls were involved in the hexavalent chromium binding and reduction process. The adsorption mechanism was preferential sequestration and binding of hexavalent chromium to ligating groups present in the biomass, followed by reduction to the trivalent state (Sanghi et al. 2009).

The foregoing indicates that living and dead fungal cells play an important role in the adsorption of heavy metals. In fact, using inactive dead cells presents several advantages: (1) treatment system effectiveness is not limited by the toxic effects on the fungi, (2) neither nutrients nor growth factors are needed, and (3) the adsorbed ions are easily recovered and reused from the biomass.

In addition, immobilization of fungal biomass within the polymeric matrix has several advantages such as ability to separate solid biomass from the bulk liquid, recovery of metals, control of particle size, fast growth and multiplication, low density level, high separation ability, low cost application of microbial absorbents and a high biomass loading (Vijayaraghavan and Yun 2008). Reya Issac et al. (2012) reported that the material used for immobilization should be rigid, chemically inert and cheap, with high loading capacity and increasing diffusion. Liu et al. (2012) found that 3% polyvinyl alcohol and 3% sodium alginate produced the most stable and efficient biobeads of *Rhizopus* sp. LG04. The most suitable matrix for *Phanerochaete chrysosporium* was reported to be Ca-alginate (Murugavelh and Mohanty 2013b). Liu et al. (2012) reported that immobilized living cells for Cr(VI) removal have the advantages of being stable, adequate for long-term treatment, easy to re-use and less biomass leakage, in comparison with free cells.

Mycorrhiza represent a symbiotic association between a fungus and the roots of a vascular plant. In a mycorrhizal association, the fungus colonizes the host plant's roots, either intracellularly as in arbuscular mycorrhizal fungi (AMF), or extracellularly as in ectomycorrhizal fungi. Such mycorrhizal associations are an important component of soil life and soil chemistry. The principal role of mycorrhizal fungi is to improve the uptake of phosphorus and mineral nutrients for plants and enhance the number of roots and length of root branches. AMF can be used to facilitate phytoremediation and the growth of plants in metal-contaminated soils (Gamalero et al. 2009; Miransari 2011). Bioremediation using mycorrhiza is termed mycorrhizoremediation (Khan 2006). Estaún et al. (2010) reported that plants inoculated with the AMF *Glomus intraradices* (BEG 72) in moderately contaminated soils, perform (i.e., in terms of growth and survival rate) as well as non-inoculated plants in soil without chromium. This suggests a buffering effect of the AMF that results in decreased uptake of the toxic element by roots and its translocation to the shoot. However, the mechanisms by which AMF alleviates phytoremediation of metals is not clear (Karami and Shamsuddin 2010).

3.4 Algae

Algae are photosynthetic organisms. Both growing and non-living algal cells are capable of removing Cr(VI). The first step involved in the binding of Cr(VI) ions to algal species is binding to the cell surface. This process occurs rapidly and is independent of cellular metabolism. The second step of intracellular accumulation of a metal results from a simultaneous growth and surface biosorption effects. This step requires cell metabolic energy and is a much slower process (Sen and Ghosh Dastidar 2010).

For *Chlorella miniata* (Han et al. 2007) and the green algae *Cladophora albida* (Deng et al. 2009), biosorption of Cr(VI) occurred first, followed by bioreduction of Cr(VI) and biosorption of Cr(III) onto the algal biomass.

Among different types of biological material, algae have several advantages, i.e., they can be economically regenerated, the metal can potentially be recovered, less biological sludge is generated, algal material works at high efficiency in dilute effluents and has a large surface area to volume ratio (Gupta et al. 2009).

3.5 Genetically Engineered Microorganisms (GEM)

There are numerous approaches for increasing the efficiency of bacterial bioremediation. The first method is to promote bacterial growth by providing nutrients that favor a specific species, which allows it to out compete the natural bacteria present in the environment. This approach is called biostimulation. The second method is to introduce specific competent strains or consortia of microorganisms. This approach is named bioaugmentation. Another approach is to genetically engineer microorganisms to enhance their removal abilities (Tahri Joutey et al. 2013a).

Ackerley et al. (2004) reported that genetic and protein engineering of suitable enzymes can improve bacterial bioremediation. Ackerley et al. (2004) described ChrR as a dimeric flavoprotein that catalyzes the reduction of Cr(VI) optimally at 70 °C. An open reading frame, yieF, on the *E. coli* chromosome with no assigned function was found to have a high homology to chrR. This gene was cloned and the encoded protein, YieF, showed maximum reduction of Cr(VI) at 35 °C (Park et al. 2002). Recently, Frederick et al. (2013) engineered bacteria to produce trehalose and found that they then reduced 1 mM Cr(VI) to Cr(III), whereas wild-type cells were only able to reduce half that amount. They concluded that by providing bacteria with a biochemical defense against the side-effects of chromate, reduction may be a new approach for cleaning up sites that are contaminated with high levels of chromate (Frederick et al. 2013). Rajamani et al. (2007) developed transgenic approaches that enhanced the heavy metal specificity and binding capacity of microalgae for efficient heavy metal phytoremediation of contaminated wastewaters and sediments. The transgenic strategies include over expression of enzymes whose metabolic products ameliorate the effects of heavy metal-induced stress, and the expression of high-affinity, heavy-metal binding proteins on the surface and in the cytoplasm of transgenic cells.

Genetic engineering may also be utilized for more comprehension of the genetic basis of Cr(VI) resistance and its reduction. Using plasmid transfer and curing studies, Verma et al. (2009) reported that both chromate resistance and reduction were plasmid mediated and *Bacillus brevis* harbored a stable 18 kb plasmid DNA. GEMs may have higher activity in transforming metals. However, there is considerable controversy surrounding the release of such GEMs into the environment. Therefore, field testing of these organisms must be delayed until the human and environmental can be assured. Although this issue has been addressed by many regulatory agencies and scientists, no single set of guidelines with universal acceptance is presently available (Tahri Joutey et al. 2013a).

4 Resistance Mechanisms

The majority of microbial species are sensitive to Cr(VI), but some species are resistant and can tolerate high levels of chromate. In bacteria, Cr(VI) resistance is mostly plasmid borne, whereas Cr(VI) reductase genes are found both on plasmids

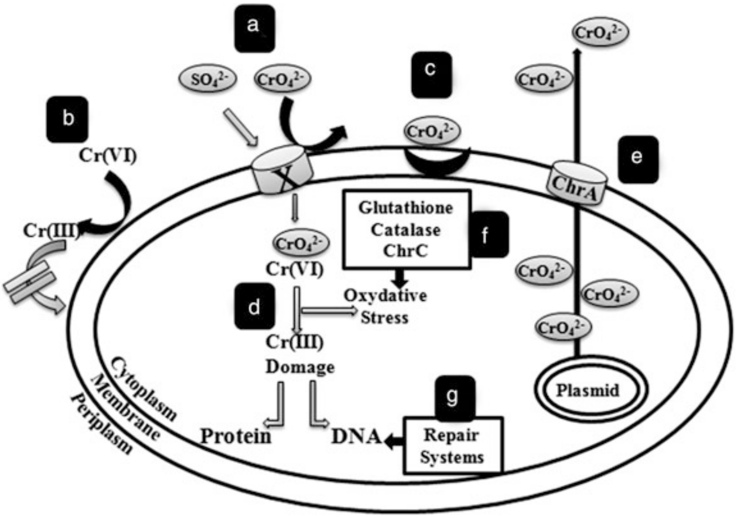

Fig. 1 A schematic depicting the mechanisms of microbial chromate transport, toxicity, resistance and reduction. (**a**) Sulfate uptake pathway, which is also used by chromate to enter cells. (**b**) Extracellular reduction of Cr(VI) to Cr(III), in which the metal forms do not cross the membrane. (**c**) Membrane-bound chromate reductase. (**d**) Intracellular Cr(VI) to Cr(III) reduction may generate reactive oxygen species (ROS) and thereby oxidative stress that causes protein and DNA damage. (**e**) Active efflux of chromate from the cytoplasm by means of the ChrA protein. (**f**) Detoxifying enzymes can be exuded to protect against oxidative stress. (**g**) DNA repair systems protect against damage generated by chromium derivatives (Modified from Ramirez-Diaz et al. (2008))

and on the main chromosome. The best characterized mechanisms comprise efflux of chromate ions from the cell cytoplasm and reduction of Cr(VI) to Cr(III) (Ramirez-Diaz et al. 2008). Chromate-resistant as well as chromate-sensitive bacterial isolates are able of reducing Cr(VI), which capability may relate to the involvement of chromate reductase activity. However, many organisms possess chromate resistance from the presence of an effective efflux mechanism (Thacker et al. 2006).

Several mechanisms have been described to account for bacterial resistance to chromate (Fig. 1). These include the following:

- Ability to regulate uptake mechanisms such as the sulfate uptake shuttle system that is involved in initial cellular accumulation (Brown et al. 2006).
- Extracellular capacity to reduce Cr(VI) to Cr(III), which is then removed easily by via reactions with functional groups on bacterial cell surfaces (Ngwenya and Chirwa 2011).
- Capacity to reduce Cr(VI) to Cr(III) in the cell membrane, usually preceded by the adsorption of Cr(VI) to functional groups that are located on the bacterial cell surface (Opperman and van Heerden 2008; Tahri Joutey et al. 2013b).

- Intracellular reduction of Cr(VI) to Cr(III) and salting out of Cr(III) to the exterior of cells. The intracellular reduction of Cr(VI) keeps the cytoplasmic concentration of Cr(VI) low and facilitates accumulation of chromate from the extracellular medium into the cell.
- Ability to counter chromate-induced oxidative stress induced by activating enzymes that are involved in ROS scavenging (e.g., catalase, superoxide dismutase) (Ackerley et al. 2006; Cervantes and Campos-García 2007).

Flora (2009) reported that antioxidant enzymes and non-enzymatic antioxidants (e.g., vitamin C and E, carotenoids, thiol antioxidants and flavonoids) are known to counteract the effect of ROS. These antioxidants are known to diffuse free radicals and limit the risk of oxidative stress. At the cellular and molecular level antioxidants inactivate ROS, and at low concentrations inhibit or delay oxidative processes by interrupting the radical chain reaction. Antioxidants also chelate the metal ions responsible for generating ROS.

- Presence of an efflux system, which is the most common mechanism of plasmid-controlled bacterial metal ion resistance.
- Specialized repair of DNA damage by SOS response enzymes (RecA, RecG, RuvAB) (Hu et al. 2005; Cervantes and Campos-García 2007).
- Ability to regulate iron uptake, which may serve to sequester iron and prevent the generation of highly reactive hydroxyl radicals via the Fenton reaction (Brown et al. 2006).

The best characterized mechanisms comprise efflux of chromate ions from the cell cytoplasm, reduction of Cr(VI) to Cr(III) and chromium uptake and are discussed below.

4.1 *Efflux Mechanism*

Alvarez et al. (1999) reported plasmid-determined resistance to chromate ions in the genera *Streptococcus, Pseudomonas* and *Alcaligenes*. The molecular analysis of chromate resistance determinants from plasmid pUM505 of *Pseudomonas Aeruginosa* and plasmid pMOL28 of *Alcaligenes eutrophus* revealed that the deduced product of the chrA gene, hydrophobic protein ChrA (416 and 401 amino acid residues, respectively) was responsible for the resistance phenotype. Chromate tolerance conferred by the ChrA protein was associated with reduced accumulation of CrO_4^{2-} in both *P. aeruginosa* and *A. eutrophus,* and it was hypothesized that ChrA was involved in the extrusion of chromate ions. Nevertheless, direct evidence for efflux was missing. Alvarez et al. (1999) showed that the membrane vesicles from chromate-resistant *P. aeruginosa* cells that expressed the ChrA protein accumulated four-fold more CrO_4^{2-} than did vesicles prepared from a plasmidless chromate-sensitive derivative, indicating that a chromate efflux system functions in the resistant strain. They also reported that uptake of chromate by vesicles was dependent on nicotinamide adenine dinucleotide (NADH) oxidation and was abolished by energy inhibitors and by the chromate analog sulfate (Alvarez et al. 1999).

Juhnke et al. (2002) reported that *Cupravidus metallidurans* and *P. aeruginosa* have served as model organisms for chromate efflux occurring via the ChrA protein, and produced resistance levels of 4 and 0.3 mM, respectively. However, chromate efflux has only been biochemically identified as a resistance mechanism in *Proteobacteria* (Branco et al. 2008). Branco et al. (2008) reported that the highly tolerant strain *Ochrobactrum tritici* 5bvl1 survived chromate concentrations of >50 mM and have the transposon Tn*OtChr,* which contains a group of *chrB*, *chrA*, *chrC* and *chrF* genes. The *chrB* and *chrA* genes, but not *chrF* or *chrC*, were essential for establishing high resistance in chromium-sensitive *O. tritici*. They also reported that, the *chr* promoter was strongly induced by chromate or dichromate, but it was completely unresponsive to Cr(III), oxidants, sulfate, or other oxyanions. Induction of the *chr* operon suppressed accumulation of cellular Cr through the activity of a chromate efflux pump that is encoded by *chrA* (Branco et al. 2008).

The CHR protein family, which includes putative ChrA orthologs, currently contains about 135 sequences from all three life domains (Ramirez-Diaz et al. 2008). There is considerable variation in the genomic context surrounding ChrA orthologs (Diaz-Perez et al. 2007), which raises the question as to whether functional or regulatory differences in chromate efflux among organisms bearing ChrA orthologs also exist. Although the CHR superfamily includes representatives from all domains of life, at the time of its construction, the phylogeny was largely dominated by Proteobacteria (35 out of 72 organisms). Moreover, given the high levels of chromate resistance among Actinomycetales such as *Arthrobacter*, the 135 ChrA orthologs (which includes only three representatives within the order Actinomycetales: *Corynebacterium glutamicum, C. efficiens* and *Kineococcus radiotolerans*) reported by Ramirez-Diaz et al. (2008) probably underestimates the range of this protein family, suggesting that the family warrants further investigation.

Recently, the *Lysinibacillus fusiformis* ZC1 strain was found to contain large numbers of metal resistance genes, such as the chrA gene, which encodes a putative chromate transporter that confers chromate resistance. A yieF gene and several genes encoding reductases that were possibly involved in chromate reduction were also found; moreover, the expression of two adjacent putative chromate reduction-related genes, nitR and yieF, was regarded to be constitutive (He et al. 2011).

As a structural analog of sulfate (SO_4^{2-}), chromate enters cells through sulfate uptake systems. If the bacteria possess intracellular chromate reductases, Cr (VI) will be reduced to Cr(III). If not, Cr(VI) accumulated inside the cell induces the *chr* operon and activates the chromate efflux pump that is encoded by *chrA*. Therefore, the bacterial cell is protected from Cr(VI) toxicity by being repulsed outside the cell (Fig. 2).

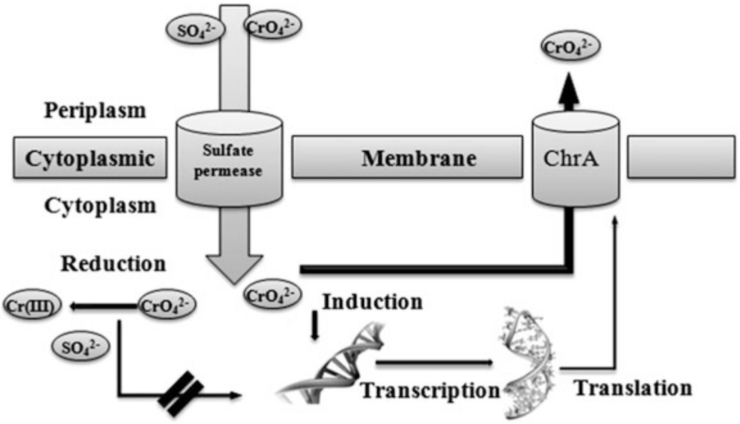

Fig. 2 Mechanisms by which microorganisms become resistant via intracellular Cr(VI) reduction to Cr(III) and its ejection by the efflux system

4.2 Reduction of Chromate

Bacteria reduce Cr(VI) by chemical (indirect) or enzymatic (direct) means. The chemical reduction of Cr(VI) involves compounds like cysteine, glutathione, sulfite and thiosulfates (Donati et al. 2003). The enzymatic reduction of Cr(VI) is achieved by soluble and membrane-bound reductases that exist in a diverse range of aerobic, facultative and anaerobic bacteria (Ramirez-Diaz et al. 2008). Under anaerobic conditions, biological reduction is slow, so abiotic reduction by Fe(II) or hydrogen sulfide tends to be the dominate process (Somasundaram et al. 2009). Microbial reduction only becomes kinetically important in aerobic environments. In anaerobic bacteria, chromate reduction generally occurs in the presence of membrane-bound enzymes. In contrast, enzymes that reduce chromate are localized as soluble cytosolic proteins in most aerobic bacteria (Puzon et al. 2002).

Chromate reduction is not typically considered to be a resistance mechanism (Cervantes and Silver 1992), hence, chromate reduction and resistance are independent processes (Verma et al. 2009). Cr(VI) reduction mechanisms and localization will be discussed in details below.

4.3 Cr(VI) Uptake

Bioaccumulation includes all processes responsible for the uptake of available metal ions by living cells. It includes biosorption, and intracellular accumulation and bioprecipitation mechanisms (Tripathi and Garg 2013). Hexavalent chromium ions can become entrapped in cellular structures and subsequently biosorbed onto the binding sites therein. Such uptake does not require energy and is termed biosorption or passive uptake. Cr(VI) also penetrates cell membranes in ways that require metabolic energy input. Such membrane transmission is termed active uptake. Both active and passive modes of metal uptake may lead to bioaccumulation of the absorbed metal (Iyer et al. 2004).

4.3.1 Biosorption of Chromium

Biosorption can be used to remove pollutants from waters, especially those that are not easily biodegradable such as metals. Many researchers have developed sorption-based processes that employ synthetic resins, activated carbons, inorganic sorbent materials, or the so-called biosorbents derived from nonliving biomaterials. Of these, biosorbents are generally the cheapest, most abundant and environmentally friendly option (Park et al. 2008). A variety of biomaterials are known to bind pollutants, including nonliving bacteria, fungi, algae, seaweed, industrial byproducts and agricultural wastes (Mohan and Pittman 2006).

According to Saha and Orvig (2010), there are four different Cr(VI) biosorption mechanisms:

1. Anionic adsorption to cationic functional groups: Negatively charged chromium species (chromate (CrO_4^{2-})/dichromate ($Cr_2O_7^{2-}$) in the medium) bind via electrostatic attraction to positively charged functional groups on the surface of biosorbents. This mechanism is based on the observation that at low pH, Cr (VI) adsorption increases and at high pH, Cr(VI) adsorption decreases. Indeed, at low pH functional groups of the biosorbent become protonated and easily attract negatively charged chromium. In contrast, at high pH deprotonation occurs, functional groups become negatively charged, repelling negatively charged chromium. Garg et al. (2013) revealed that functional groups like carbonyl and amide of bacterial cells might be involved in adsorping reduced Cr(III) on the surface of *P. putida*.
2. Adsorption-coupled reduction: In this mechanism, Cr(VI) is totally reduced to Cr(III) by biomass in the presence of an acid, which then is adsorbed to the biomass. The amount of adsorption depends on the nature of the biosorbent (Sanghi et al. 2009).
3. Anionic and cationic adsorption: In this mechanism, a portion of Cr(VI) is reduced to Cr(III). The anionic and cationic [Cr(VI) and Cr(III)] forms are then adsorbed to biosorbents.
4. Reduction and anionic adsorption: Herein, a portion of the Cr(VI) is reduced to Cr(III) by a biosorbent, and mainly Cr(VI) is adsorbed to the biomass, whereas Cr(III) remains in the solution.

4.3.2 Bioaccumulation of Chromium

Biological membranes are practically impermeable to Cr(III). However, Cr(III) readily forms complexes in aqueous solution with most biologically relevant ligand molecules and these complexes may be taken up by cells (Ksheminska et al. 2005). Cr(VI) exists mainly as the tetrahedral CrO_4^{2-}, and this form is analogous to physiological anions such as SO_4^{2-} and PO_4^{2-}. Cr(VI) enter cells via facilitated transport through a non-selective anion channel, or through sulfate transporters, in which competition exists between Cr(VI) and sulfate. Therefore, sulfate supplementation relieves chromate toxicity. Additionally, Pereira et al. (2008) found that chromate strongly decreased sulfate assimilation and sulfur metabolite pools, suggesting that cells experience sulfur starvation. Cr(VI) is rapidly reduced to Cr (III) inside cells, and therefore, the concentration of Cr in the Cr(VI) oxidation state will never be equal on both sides of a plasma membrane (as long as the cells have a satisfactory reducing capacity). Reduction capacity of the cells is the main power by which Cr(VI) is bioaccumulated.

Several papers have described how living and dead microbial cells have been applied to remove Cr(VI) from water solutions by biosorption (Mungasavalli et al. 2007; Anjana et al. 2007) and bioaccumulation (Ksheminska et al. 2005; Srivastava and Thakur 2006). Recently, Long et al. (2013) isolated *Pseudochrobactrum asaccharolyticum* LY6, a species that had not previously been reported to remove Cr(VI). Transmission electron microscopy and energy dispersive X-ray spectroscopy (TEM-EDS) analysis further confirmed that strain LY6 could accumulate chromium within the cell while removing Cr(VI). Each removal method has advantages and disadvantages. Applying dead biomass solves limitations associated with metal toxicity and maintenance of cell metabolic activity. Furthermore, the adsorbed metal may be easily collected and the biomass may be reused. However, this method is limited by the fact that no reactions proceed in dried cells. The application of living biomass allows metal to be removed as microbes grow, and avoids microorganismal reproduction, biomass drying and storage. Unfortunately, when using living biomass, if the metal concentration in the environment is too high, it may be toxic to the growing biomass. Therefore, when possible, microorganisms should be applied that have high tolerance to high Cr(VI) concentrations, or should be pre-adapted to the toxicant (Hołda et al. 2011).

5 Cr(VI) Reduction Mechanisms and Localization

Cr(VI) reduction may be cometabolic (not participating in energy conservation) in certain bacterial species, but could be predominantly dissimilatory/respiratory under anaerobic conditions in other species. Under anaerobic conditions, Cr (VI) serves as a terminal electron acceptor in the membrane electron-transport respiratory pathway, a process resulting in energy conservation for growth and cell maintenance. In the dissimilatory/respiratory process, NADH donates electrons to Cr(VI) (Chirwa and Molokwane 2011).

Several enzymatic Cr(VI) reduction types exist in bacteria; such enzymes include Cr(VI) reductase, aldehyde oxidase, cytochrome P450, and DT-diaphorase (Patra et al. 2010). Similarly, several oxidoreductases with different metabolic functions have also been reported to catalyze Cr(VI) reduction in bacteria, such as nitroreductase (Kwak et al. 2003), hydrogenases (Chardin et al. 2003), iron reductase and quinone reductases (Gonzalez et al. 2005), flavin reductases (Puzon et al. 2002; Ackerley et al. 2004), and NADH/NADPH-dependent reductases (Bae et al. 2005).

The enzymatic reduction of Cr(VI) utilizes membrane-bound chromate reductase during anaerobic respiration or employs a soluble cytosolic chromate reductase under aerobic conditions, the activity of which is enhanced by NADH or glutathione as enzyme co-factors (Elangovan et al. 2006). In such processes chromate acts as the terminal electron acceptor.

5.1 Direct Cr(VI) Reduction

5.1.1 Aerobic Cr(VI) Reduction

As shown in Fig. 3, bacterial Cr(VI) reduction in the presence of oxygen occurs as a two or three step process, with Cr(VI) initially reduced to the short-lived intermediates Cr(V) and/or Cr(IV) before being further reduced to the thermodynamically stable end product, Cr(III). Cr(V) undergoes a one-electron redox cycle to regenerate Cr(VI) by transferring the electron to oxygen. This process produces a ROS that easily combines with DNA–protein complexes. Nevertheless, it is presently unclear whether the reduction of Cr(V) to Cr(IV) and Cr(IV) to Cr(III) is spontaneous or enzyme mediated (Cheung and Gu 2007). NADH, NADPH and electrons from the endogenous reserve are implicated as electron donors in the Cr (VI) reduction process. Reductases (viz., ChrR, YieF and Tkw3) reduce Cr (VI) species by shuttling electrons to form Cr(III) (Qamar et al. 2011).

Aerobic Cr(VI) reduction is generally associated with soluble proteins utilizing NADH as an electron donor, either as a requirement or to enhance activity (Elangovan et al. 2006). Several researchers have reported chromate reductase activity in cell-free extracts during aerobic Cr(VI) reduction (Rida et al. 2012; Tripathi and Garg 2013).

5.1.2 Anaerobic Cr(VI) Reduction

In the absence of oxygen, Cr(VI) can serve as a terminal electron acceptor in the respiratory chain for a large array of electron donors, including carbohydrates, proteins, fats, hydrogen, NAD(P)H and endogenous electron reserves. Both soluble and membrane-associated enzymes have mediated the process of Cr(VI) reduction under anaerobic conditions (Cheung and Gu 2007). Unlike Cr(VI)-reductases isolated from aerobes, the Cr(VI)-reducing activities of anaerobes are associated with their electron transfer systems ubiquitously catalyzing the electron shuttle along the respiratory chain. Furthermore, the cytochrome family (e.g., cytochrome

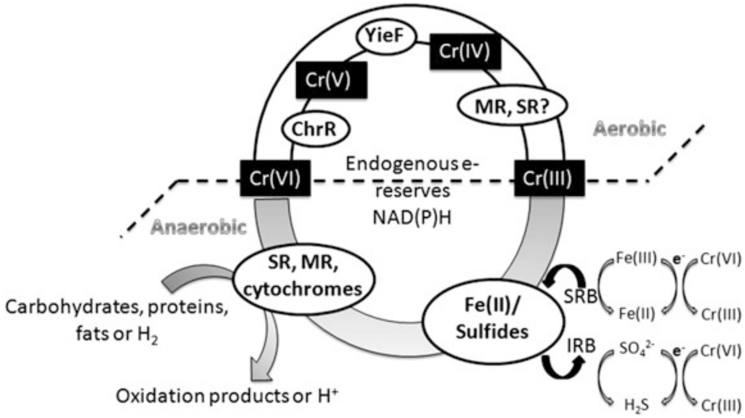

Fig. 3 Mechanisms of Cr(VI) reduction to Cr(III) in bacteria. Under aerobic conditions, NAD(P)H and endogenous e- reserves are implicated as electron donors for Cr(VI) reduction by reductases like ChrR and YieF. Under anaerobic conditions, both soluble (SR) and membrane-associated (MR) enzymes mediate Cr(VI) reduction. Metabolites of some anaerobes such as H_2S (produced by SRB and Fe(II) by IRB) are effective Cr(VI) reductants

b and c) is frequently involved in enzymatic anaerobic Cr(VI) reduction (Mangaiyarkarasi et al. 2011). Furthermore, as explained earlier, natural anaerobe metabolites, such as H_2S that are produced by sulfate-reducing bacteria and Fe (II) formed by iron reducing bacteria, are effective indirect chemical Cr (VI) reductants under anoxic environmental conditions (Cheung and Gu 2007).

5.2 *Indirect Cr(VI) Reduction via Iron- and Sulfate-Reducing Bacteria*

Sulfate- and iron-reducing bacteria (SRB and IRB) are important members of anaerobic microbial communities, and they have attracted economic, environmental and biotechnological interest. The reduction of Cr(VI) by biogenic Fe(II) and sulfides that are generated by IRB and SRB occurs 100 times faster than by CRB alone. As shown in Fig. 3, SRB produces H_2S, which serves as a Cr(VI) reductant and involves three stages: (a) reduction of sulfates, (b) reduction of chromate by sulfides and (c) precipitation of Cr(VI) by sulfide. The reduction of Cr(VI) by Fe (II) occurs when IRB reduces Fe(III) to Fe(II), which in turn reduces Cr(VI) to Cr (III) (Viti and Giovannetti 2007; Somasundaram et al. 2011).

5.3 *Extracellular Cr(VI) Reduction*

Two pathways of Cr(VI) reduction have been suggested for gram-negative bacteria (Chirwa and Molokwane 2011). The first mechanism suggests that the reduction of Cr(VI) is mediated by a soluble reductase, with NADH serving as the electron donor, either by necessity or to achieve maximum activity. The NADH-dehydrogenase pathway is expected to predominate under aerobic conditions.

The Cr(VI) reducing enzymes or soluble Cr(VI) reductases that are produced deliberately by the cell and exported into the media to reduce Cr(VI) are of special interest. Since protein excretion is an energy intensive process, most of these enzymes are produced constitutively, i.e., they are produced only when Cr(VI) is detected in solution and are therefore highly regulated (Cheung and Gu 2007). Extracellular Cr(VI) reduction is beneficial to the organism in that the cell does not require transport mechanisms to carry the chromate and dichromate into the cell, and to later export the Cr(III) into the medium. Both Cr(VI) and Cr(III) react easily with DNA, the presence of which can result in DNA damage and increased rates of mutations. Hence, extracellular reduction of Cr(VI) protects the cell from the DNA damaging effects of Cr(VI). It may be for this reason that certain bacterial species have adopted the extracellular Cr(VI) reduction process for survival in Cr (VI) contaminated environments.

From an engineering perspective, using cells that reduce Cr(VI) externally is particularly beneficial, because they allow the cells to be easily separated from an expired medium and then reused in the reactor system. Furthermore, if Cr(VI) is reduced internally, the resulting Cr(III) will tend to accumulate inside the cell, making it difficult to recover the reduced chromium or to regenerate the cells (Chirwa and Molokwane 2011).

5.4 *Membrane-Bound Cr(VI) Reduction*

Cr(VI) acts as an electron acceptor in a process mediated by a membrane-bound Cr (VI) reductase, which is active in respiratory chains that involve cytochromes (Wang et al. 1991).

A membrane-associated chromate reductase from *Thermus scotoductus* SA-01 has been purified to apparent homogeneity, and has been shown to couple the reduction of Cr(VI) to NAD(P)H oxidation, with a preference towards NADH. Sequence homology identified the protein as a dihydrolipoamide dehydrogenase, which is part of the multi-subunit pyruvate dehydrogenase complex (Opperman and van Heerden 2008). A chromate reductase assay from the alkaliphilic gram-positive *Bacillus subtilis* indicated that the Cr(VI) reduction was mediated by constitutive membrane-bound enzymes, and a decrease in pH with growth of the bacterium signified the role played by metabolites (organic acids) in chromium resistance and reduction mechanism (Mangaiyarkarasi et al. 2011). Tahri Joutey et al. (2013b) reported that the membrane-associated chromate reductase activity of *S. proteamaculans* is constitutive and is preceded by its adsorption on the cell surface.

5.5 Intracellular Cr(VI) Reduction

Although it has been demonstrated that specialized Cr(VI) reducing enzymes (reductases) exist inside Cr(VI)-reducing bacterial cells, several components of the cell's protoplasm also reduce Cr(VI). Components such as NADH (NADPH in some species), flavoproteins and other hemeproteins readily reduce Cr(VI) to Cr (III) (Ackerley et al. 2004). It is therefore expected that the cytoplasm fraction of disrupted cells from most organisms will reduce Cr(VI). Such a reduction process is not energy consuming but will directly affect the cell, since most of the intracellular proteins catalyze a one-electron reduction from Cr(VI) to Cr(V). When this occurs, harmful reactive-oxygen species (ROS) are generated that cause damage to DNA.

Hexavalent chromate reductase was found to be localized in the cytoplasmic fraction of several chromium-resistant bacteria, e.g., *Bacillus cereus* (Iftikhar et al. 2007) and *Pannonibacter phragmitetus* LSSE-09 (Xu et al. 2012). In contrast, bacteria like *Pseudomonas putida* (Garg et al. 2013) and *Bacillus cereus* (Tripathi and Garg 2013) displayed chromate reductase activity that was mainly associated with both the supernatant and cytosolic fractions of bacterial cells.

6 Summary

Chromium has been and is extensively used worldwide in multiple industrial processes and is routinely discharged to the environment from such processes. Therefore, this heavy metal is a potential threat to the environment and to public health, primarily because it is non-biodegradable and environmentally persistent. Chromium exists in several oxidation states, the most stable of which are trivalent Cr(III) and hexavalent Cr(VI) species. Each species possesses its own individual chemical characteristics and produces its own biological effects. For example, Cr (III) is an essential oligoelement for humans, whereas Cr(VI) is carcinogenic and mutagenic. Several chemical methods are used to remove Cr(VI) from contaminated sites. Each of these methods has advantages and disadvantages. Currently, bioremediation is often the preferred method to deal with Cr contaminated sites, because it is eco-friendly, cost-effective and is a "natural" technology.

Many yeast, bacterial and fungal species have been assessed for their suitability to reduce or remove Cr(VI) contamination. The mechanisms by which these microorganisms resist and reduce Cr(VI) are variable and are species dependent. There are several Cr-resistance mechanisms that are displayed by microorganisms. These include active efflux of Cr compounds, metabolic reduction of Cr(VI) to Cr

(III), and either intercellular or extracellular precipitation. Microbial Cr (VI) removal typically involves three stages: binding of chromium to the cell surface, translocation of chromium into the cell, and reduction of Cr(VI) to Cr (III). Cr(VI) reduction by microorganisms may proceed on the cell surface, outside the cell, or intracellularly, either directly via chromate reductase enzymes, or indirectly via metabolite reduction of Cr(VI). The uptake of chromium ions is a biphasic process. The primary step is known as biosorption, a metabolic energy-independent process. Thereafter, bioaccumulation occurs, but is much slower, and is dependent on cell metabolic activity. Choosing an appropriate bioremediation strategy for Cr is extremely important and must involve investigating and understanding the key mechanisms that are involved in microbial resistance to and removal of Cr(VI).

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Street Dust: Implications for Stormwater and Air Quality, and Environmental Management Through Street Sweeping

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Contents

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1 Introduction

Street dust is composed of particles that arise from motor vehicles (e.g., tire debris, emission-related particulates), local soils, and road pavement (Yeung et al. 2003). These materials are commingled with larger debris, including discarded trash, lawn clippings, fallen leaves and branches, and other detritus (Fig. 1). The nature and composition of street dust is expected to vary widely based on local climate, geology, population and traffic density, infrastructure, and other factors. Maintenance sand or road salt used in inclement weather is a source of primary granular material and can contribute to street dust through mechanical abrasion of the road surface (Kupiainen et al. 2003; Gertler et al. 2006. Kuhns et al. 2003; USGS 2013).

High levels of organic and inorganic contaminants in street dust represent a source of dual potential risk to stormwater and air quality. For example, runoff of street dust to local watersheds can degrade water quality and impact sediment (Buckler and Granato 1999; Sartor and Boyd 1972; Walker et al. 1999). It is also clear that many contaminants exist at higher concentrations in the smallest particles, which are most likely to be mobilized by runoff (Breault et al. 2005; Herngren et al. 2006; Zhao et al. 2009a). Further, studies have indicated that as much as 85 %

Fig. 1 Bulk materials gathered through street sweeping in Waco, Texas, USA

of ambient airborne particulate matter (PM_{10}), exposure to which is associated with several adverse health effects, can arise from accumulated street dust (Amato et al. 2010a).

Many large municipalities coordinate the sweeping of streets on a regular basis, and these efforts are at least partly driven by concerns about the impacts of stormwater runoff to the local environment (Brinkmann and Tobin 2001). Street cleaning is considered to be a best management practice (BMP) for storm water management by the U.S. Environmental Protection Agency (USEPA 2012). A number of different strategies and technologies are available to manage and collect street dust, including flushing, mechanical broom, vacuum and regenerative air systems. The mass of materials diverted from stormwater runoff by street cleaning can be significant. For example, more than 2,000 tons of materials per year are removed by street cleaning in the city of Waco, Texas, USA, which correspondingly has maintained a model compliance record for stormwater management (City of Waco Utilities, personal communication). Furthermore, street cleaning is increasingly studied to determine its utility in improving ambient air quality (Amato et al. 2010a, b).

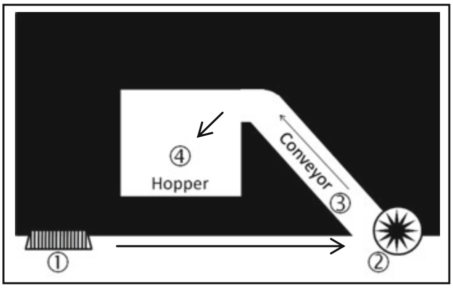
The purpose of this study was to critically review and summarize the available data on the effectiveness of various street cleaning technologies and practices for improvement of stormwater and air quality. As such, we examined the available literature that addressed street dust and its potential impacts on stormwater and air quality, and related the relative efficacy of multiple street sweeping technologies to the context of environmental/ecological and human health risk. During this exercise, 1,187 journal articles from the peer reviewed literature were compiled by searching for the following terms: “street dust,” “road dust,” “urban dust,” “roadway sediments,” etc. Of these, 89 papers contained the phrases “street sweeping,” “street cleaning,” “road sweeping,” or “road cleaning.” Only two peer-reviewed articles provided quantitative data on the comparative efficacy of multiple sweeping technologies on gathering street dust (Amato et al. 2010a; Tobin and Brinkmann 2002). Unfortunately, authors of many studies failed to sufficiently describe the specific street cleaning apparatus used in the studies they performed. In several government documents from the United States and Canada, there were attempts to assess the comparative efficacy of street cleaning across available technologies. To date, no published journal articles or government reports have characterized comparative margins of safety and/or relative risk to human health and the environment, in the context of multiple modern street sweeping technologies and strategies for street dust management. In Table 1, we summarize the available literature for which street cleaning technologies and constituents have been measured.

Most modern street sweepers fall into one of three categories: mechanical, vacuum, and regenerative air (Fig. 2). The majority (about 41 %) of sweepers in the United States are mechanical broom sweepers (Schilling 2005a, b). These sweepers remove debris with a large rotary brush fitted at the rear of the sweeper that directs collected material onto a conveyor. This conveyor leads to the hopper, where collected materials are stored until disposal. Mechanical sweepers are

Table 1 A summary of published scientific studies related to street cleaning

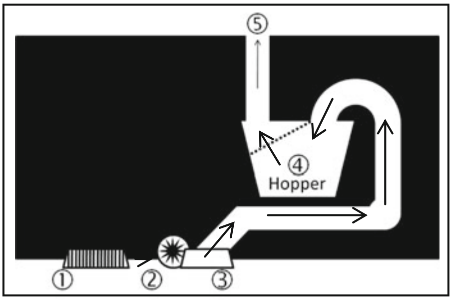
		Sweeper technology						Constituents analyzed						
		Standard			High efficiency									
Reference	Study type	Mechanical	Vacuum	Regen. air	Mechanical	Vacuum	Regen. air	Metals	Organics	Solids	Nutrients	Particle size	PM10	PM2.5
Amato et al. (2009)	Empirical		×					×					×	
Amato et al. (2010a)	Simulated	×	×	×						×		×	×	
Amato et al. (2010b)	Empirical		×					×					×	
Bender and Terstreip (1984)	Empirical	×						×		×	×			
Berretta et al. (2011)	Empirical	Unspecified								×	×			
Brinkmann and Tobin (2001)	Empirical	×		×				×	×	×	×			
Bris et al. (1999)	Empirical							×	×	×				
Calabro (2010)	Empirical	×						×		×		×		
Chang et al. (2005)	Empirical			×			×						×	
DeLuca et al. (2012)	Empirical						×						×	
ETV (2011, 2012a, b, c)	Empirical				×		×						×	×
Gasperi et al. (2005)	Empirical								×					
German and Svensson (2002)	Empirical		×					×				×		
Gertler et al. (2006)	Empirical	×											×	×
Gromaire et al. (2000)	Empirical							×		×				
Horwatich and Bannerman (2009)	Empirical		×									×		
Jang et al. (2009)	Empirical	Unspecified						×						
Kang et al. (2009)	Simulated	×	×	×				×		×			×	
Kuhns et al. (2003)	Empirical	×	×										×	
Law et al (2008)	Empirical		×					×		×	×	×		
Norman and Johansson (2006)	Empirical	×											×	

NRDL (1959)	Empirical	×	×										
NWRI (2007)	Empirical	×		×			×	×	×	×	×	×	
Sartor and Gaboury (1984)	Simulated	×	×					×		×	×		
Seattle Public Utilities and Herrera EC (2009)	Empirical			×				×	×	×	×		
Sutherland and Jelen (1997)	Simulated	×		×		×						×	
Sutherland et al. (1998)	Simulated	×		×		×		×	×	×	×	×	
Sutherland (2009)	Empirical		×	×	×		×					×	
Tandon et al. (2008)	Empirical							×			×		×
Terstriep et al. (1982)	Empirical	×						×		×	×		
Tobin and Brinkman (2002)	Empirical	×		×				×	×		×	×	
USEPA (1972)	Simulated	×	×	×				×	×	×	×	×	
USEPA (1979)	Empirical	×	×					×	×	×	×	×	×
USEPA (1983)	Simulated	Unspecified						×		×	×		
USEPA (1985a)	Empirical	×		×						×			
USEPA (1985b)	Empirical	×		×									
USGS (2002)	Empirical	×						×	×	×	×	×	
USGS (2005)	Empirical	×	×					×	×			×	
USGS (2007)	Empirical	×	×	×				×		×	×	×	
USGS (2013)	Empirical	×	×				×	×		×	×	×	
Weston Solutions (2010)	Empirical	×	×	×				×	×	×	×	×	
Wilber and Hunter (1979)	Empirical	Unspecified						×		×			
Wu et al. (2010)	Empirical		×									×	



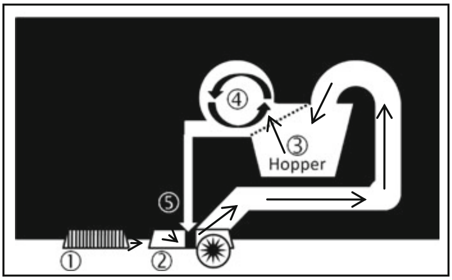
A. Mechanical Broom Sweepers

1. A rotating gutter broom directs dirt and debris from the curb into the path of a large rotating cylindrical broom.
2. The main broom flicks dirt and debris onto a conveyor.
3. The conveyor carries dirt and debris to a hopper.
4. The conveyor drops dirt and debris into a hopper.



B. Vacuum Sweepers

1. A rotating gutter broom directs dirt and debris from the curb into the path of the vacuum nozzle.
2. A windrow broom is often used to direct dirt debris into the path of the vacuum nozzle.
3. The debris-laden air stream is pulled into a hopper, at the opposite side of the suction inlet where the air loses velocity and the larger debris drops to the bottom.
4. Dirt and debris settle in hopper and lighter debris is blocked by a screen.
5. Air is exhausted from hopper.



C. Regenerative Air Sweepers

1. A rotating gutter broom directs dirt and debris from the curb into the path of the pick-up head.
2. Within the pick-up head, a blast of air dislodges and suspends dirt and debris. A broom within the pick-up head is sometimes used to dislodge stuck-on debris.
3. The debris-laden air stream is pulled into a hopper, at the opposite side of the suction inlet where the air loses velocity and the larger debris drops to the bottom.
4. Dirt passes through a centrifugal dust separator
5. Clean air returns to the blast orifice of the pick-up head.

Fig. 2 Process flow diagrams for three primary street sweeping technologies

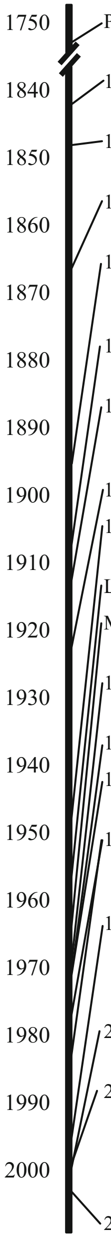
effective at picking up wet vegetation, gravel and coarse sand, but are less efficient at removing fine particles, especially those below 250 μm (Kang et al. 2009; Schilling 2005a). Vacuum street sweepers have gained popularity because of their ability to remove fine dust more effectively than mechanical sweepers USGS 2007). They use a rotary brush called a windrow boom to push dirt and debris toward the path of the suction nozzle (Fleming 1978; Sutherland 2011). Vacuum street sweepers are comparable to household vacuums, in that they suck in air with a fan, collect dust and debris, and then exhaust the air (Sutherland 2011). In general, these units have difficulty removing wet vegetation and large road debris (Schilling 2005a). The newest street sweeping technology, regenerative air, is similar to a vacuum unit, in that it uses a fan called a blower to suck in dirt and debris from the street surface; rather than just a vacuum nozzle, it uses a pick-up head with a blast orifice, which directs a strong blast of air onto the street that suspends dirt and debris with the pick-up head enclosure. Ambient air is then sucked into the hopper where larger volume forces the heavier dust and debris to fall. Some regenerative air sweepers use a centrifugal dust separator to remove the lighter dust (Schilling 2005a). Cleaned air is then returned to the blower, making it a closed-loop system with no air or dust exhausted to the atmosphere (Fleming 1978).

2 Street Cleaning, Sweeping and Mitigation

For hundreds of years street sweeping has been used as a means for municipalities to remove litter, dirt, horse droppings, and vegetation for aesthetic and sanitation purposes (Schilling 2005a; Fig. 3). Before the introduction of the mechanical sweeper, street sweeping was done manually, using a broom, shovel, and either push or horse-drawn carts (Schilling 2005a). The earliest account of street cleaning in the United States may have appeared (Fleming 1978; Michaels 1977) in Benjamin Franklin's *Autobiography*. Franklin wrote about hiring a man to sweep a paved market area in Philadelphia more than 200 years ago:

> This, for some time, gave an easy access to the market dryshod; but, the rest of the street not being paved, whenever a carriage came out of the mud upon this pavement, it shook off and left its dirt upon it, and it was soon covere'd with mire, which was not remov'd, the city as yet having no scavengers. After some inquiry, I found a poor, industrious man, who was willing to undertake keeping the pavement clean, by sweeping it twice a week, carrying off the dirt from before all the neighbour's doors, for the sum of six-pence per month, to be paid by each house (as quoted in Michaels (1977), and Fleming (1978)).

Street cleaning in New York City was conducted by the police department, until it became its own administrative branch in 1881. Colonel George E. Waring Jr. was appointed as Commissioner of Streets in 1895 (Armstrong et al. 1976; Fleming 1978; Richmann 1962; Soper 1909). Drawing from his military experience as a volunteer in the Missouri Cavalry during the Civil War, Waring outfitted street sweeping workers with white uniforms, earning them the nickname "White Wings" (Armstrong et al. 1976). Waring's efforts appeared to produce a decline in sickness, to wit, declines in diarrheal diseases and the death rate in New York City (from 26.8 per 1,000 from 1882 to 1894, to 19.6 per 1,000 in 1897) (Richmann 1962), and established New York City as a model for other cities throughout the United States (Armstrong et al. 1976).



Pre-1790: Benjamin Franklin starts street sweeping program in Philadelphia (Michaels 1977).

1843: Joseph Whitworth invents the first mechanical sweeper which also incorporated means of collection and conveyance in Manchester, England (Richmann 1962).

1849: An elevator-belt street sweeper patented in the United States. Patent #6699 (Kidwell-Ross 2012).

1868: The first self propelled sweeper for railroads is patented in the United States. Patent #79606 (Kidwell-Ross 2012).

1895: Colonel George E. Warring Jr. is appointed Commissioner of New York City Street Cleaning Department and establishes rigid standards for street cleaning (Soper 1909).

1911: The first internal combustion engine powered sweeper was shown in the United States (Richmann 1962).

1914: The first practical and commercially successful self-propelled mechanical sweeper was sold in Boise, Idaho. Invented by John M. Murphy (Richmann 1962).

1920s: Vacuum sweepers appear (Richmann 1962).

1948: The United States establishes the Federal Water Pollution Control Act (USEPA 2013a).

Late 1950s: Johnston creates the first truck mounted sweeper (Johnston 2012).

Mid 1960s: B.W. Young, founder of TYMCO, Inc. in Waco, Texas invented the regenerative air sweeper (TYMCO, Inc., personal communication, 2011).

1970: TYMCO air flow surface cleaning apparatus and air cleaning apparatus are patented (US Patents 3512206 and 3545181) (USPTO1970a, b)

1970: The United States establishes the Clean Air Act (USEPA 2013d).

1972: The United States establishes the Clean Water Act and The National Pollutant Discharge Elimination System (NPDES) (USEPA 2013a, b).

1978-1983 EPA implements The National Urban Runoff Program (NURP) - The first comprehensive study of urban stormwater pollution across the United States (USEPA 1983)

1997: South Coast Air Quality Management District (AQMD) in California adopts Rule 1186, requiring local governments within the AQMD to procure Rule 1186 PM 10 certified street sweepers for new equipment purchases or new street sweeping contracts made after January 1, 2000 (AQMD 2008).

2000: TYMCO dustless regenerative air sweeper patented (US Patent 6161250) (USPTO 2000).

2000: AQMD adopts Rule 1186.1, requiring certain public and private sweeper fleet operators to acquire alternative-fuel or otherwise less-polluting sweepers when purchasing or leasing these vehicles for sweeping operations undertaken by or for government entities in the jurisdiction of the AQMD after July 1, 2002 (AQMD 2009).

2003: The Clean Roads to Clean Air program (CRCA) was initiated by the City of Toronto and tested street sweeper technology efficiency in removing fine particulate matter (PM 10 and PM 2.5) (Toronto 2008).

Fig. 3 A timeline of usage and technological development in street sweeping

2.1 *Street Cleaning Technologies*

2.1.1 Manual

Manual cleaning represents the oldest form of street cleaning in the U.S., and is still selectively practiced today. Though it has been largely replaced by modern methods, it retains several advantages, including low initial and maintenance cost of equipment; manual push brooms can reach into places inaccessible to mechanical sweepers such as areas harboring parked cars, small alleyways, and busy city areas, no matter the time of day, level of traffic, or weather conditions (Fleming 1978).

2.1.2 Mechanical

The first mechanical sweepers were horse drawn with a rotary broom at the rear, which would push dirt from the center of the street to the curb. Dirt would then be pushed into piles, and then shoveled on carts by workers (Richmann 1962). Such horse drawn sweepers were used in New York City as early as 1882 (Armstrong et al. 1976). Because these early mechanical sweepers created a great deal of dust, tanks were added to sprinkle water onto the street. Later, more powerful high capacity sprinkling wagons would follow the sweeper (Richmann 1962).

The first mechanical sweeper that integrated collection and conveyance was invented by Joseph Whitworth and was used in Manchester, England in 1843 (Richmann 1962). In the United States, Charles B. Brooks is often credited with patenting the first elevator-belt mechanical street sweeper in 1896; however, other sources cite his system as just one of many around the same time. Over 300 US patents for street sweepers were issued prior to 1900, the earliest of which may be an elevator-belt sweeper (1849) (Kidwell-Ross 2012).

Among those systems invented prior to 1900 was the first self-propelled sweeper patented in the United States in 1868, which was intended for cleaning railroad tracks. In 1891, a self-propelled steam-powered sweeper, designed to clean roads, was also patented (Kidwell-Ross 2012). An electrically-powered street sweeper was used in Berlin, Germany in the early 1900s (Soper 1909). In 1911, the first internal combustion-engine-powered sweeper was demonstrated in the United States, but was not widely accepted because it lacked maneuverability; moreover, its pan-type shelf was only large enough to hold sweepings from one side of a single block, and its engine could not supply power to both the sweeping apparatus and provide mobility for the unit (Armstrong et al. 1976; Richmann 1962). The first practical and commercially successful self-propelled mechanical sweeper was sold in Boise, Idaho in 1914. It was invented by John M. Murphy in a partnership with American Tower and Tank Company, which would later become Elgin Sweeper Company (Armstrong et al. 1976; Fleming 1978; Richmann 1962).

Modern mechanical broom sweepers use a large rotary brush that is as wide as the sweeper, and which flicks dirt and debris onto a conveyor that then carries dirt and debris to a hopper (Schilling 2005a; Sutherland 2011). Water is often sprayed for dust suppression (Kang et al. 2009) and gutter brooms may be used to move dirt and debris from the gutter to the path of the brush (Schilling 2005a; Sutherland 2011). Mechanical sweepers remain the main type of sweeper used by municipalities, despite advances in alternative sweeper technologies (Sutherland 2011). According to a survey conducted in 2005, about 41 % of municipalities in the United States and Canada still use traditional mechanical broom sweepers, rather than vacuum and regenerative air varieties (Schilling 2005a). A survey conducted in 2001 shows that 81 % of cities having populations greater than 250,000 use mechanical sweepers, with only 5 % that use vacuum or regenerative air sweepers (Brinkmann and Tobin 2001).

Mechanical sweepers are effective at picking up wet vegetation, coarse sand, and heavy material such as gravel, but they are less efficient at removing finer particles (60 μm and smaller) left behind in cracks and uneven pavement. In a sense, mechanical sweepers may actually contribute to storm water-related pollution, because the rotary action of the broom breaks down large particles to smaller ones that are then transported by surface runoff (Schilling 2005a; Sutherland 2011). Mechanical sweepers can also increase airborne dust in dry weather despite the use of water to suppress it (Sutherland 2011). Use of too much water, of course, turns street dirt to mud, making its removal difficult.

2.1.3 Flushing

Historically, another method of removing street dust was simply to flush it into runoff drains. Early flushers used gravity to discharge water. With the development of the gasoline engine, high pressure water pumps were added to facilitate flushing. Water was sprayed with high pressure at an angle on the street that created a "chisel" action, removing stuck-on dirt and debris, which could then be washed to the curb or introduced to the stormwater system (Richmann 1962).

Street flushing is no longer common practice in the United States, because dirt was frequently flushed to the gutters rather than being removed. Water quality implications of street dust intentionally washed to streams and reservoirs also detracted from the utility of street flushing operations, as did the damage it caused to pavement (Richmann 1962). Though flushing is no longer a common practice in the United States, it remains so in other countries. For example, Amato et al. (2009) evaluated the effectiveness of flushing as a street cleaning method in Spain, Chang et al. (2005) used a street washer in Taiwan, and Bris et al. (1999) assessed the effectiveness of a water-jet cleaning procedure in France.

2.1.4 Vacuum

Vacuum sweepers were first used in the 1920s to remove fine dust left behind mechanical sweepers (Armstrong et al. 1976; Fleming 1978; Richmann 1962). A primary advantage of early vacuum sweepers is that they operated in freezing weather without water use; a disadvantage of these systems was that they were noisy and unreliable (Richmann 1962). In the early 1970s, several sweeper manufacturers reintroduced vacuum sweepers to the market, and again, their primary function was to remove fine particulate matter left behind by mechanical sweepers (Armstrong et al. 1976).

Modern vacuum sweepers use an engine powered fan to create suction. Vacuum sweepers are frequently designed with the vacuum nozzles typically placed on one side of the sweeper (Sutherland 2011) or on both sides with one suction nozzle operating at a time. Most vacuum sweepers use gutter brooms and a rotary brush called a windrow boom to push dirt and debris in the path of a suction nozzle (Fleming 1978; Sutherland 2011). An inherent problem with the windrow broom is that it tends to brush dirt and debris into street cracks. This results from pavement irregularities, which may contribute to inefficient collection of street dust across all technology types (Sutherland 2011). The abrasive nature of the brooms used may also produce finer size particles that are more easily transported by surface runoff (Schilling 2005a).

Vacuum sweepers may use a filtration system or water for dust suppression (USGS 2005; Fleming 1978; Zarriello et al. 2002). Typically, used air is exhausted from the sweeper, releasing a large amount of particulate matter into the atmosphere (Sutherland 2011). Vacuum sweepers are not as effective as mechanical sweepers when picking up wet vegetation or large debris (Schilling 2005a). Several studies have shown that vacuum sweepers are more effective than mechanical sweepers at picking up smaller particles. When comparing an Elgin Pelican (mechanical sweeper) to a Johnston 605 Series (vacuum sweeper), USGS (2005) found that the vacuum sweeper was at least 1.6–10 times more efficient than the mechanical sweeper for vacuuming all particle-size ranges. In addition, they found that vacuuming efficiencies for particle sizes between 2 mm and 250 μm (or coarse sand) were at least 1.5–5 times greater for the vacuum, than for the mechanical sweepers.

When comparing an Elgin Pelican (mechanical sweeper) to an Elgin Crosswind (regenerative air sweeper) and an Elgin Whirlwind (vacuum sweeper), USGS (2007) found that regenerative air and vacuum sweepers had similar pickup efficiencies of 25 and 30 %, compared to ~5 % of street-dirt yield for the mechanical sweeper.

2.1.5 Regenerative Air

Regenerative air sweepers were invented by a road construction contractor, B.W. Young of Young Brothers Construction Company in Waco, Texas, U.S.A. during the mid-1960s. These units were designed to sweep dirt from pavement cracks to achieve better bonding of Slurry Seal, an emulsified mixture of asphalt and sand used to repair broken pavement and restore asphalt roads (TYMCO 2012a, b, c, d).

Regenerative air sweepers use an engine to power a blower, which pushes air forward through a blast orifice across the entire width of a pick-up head onto the street surface and into the cracks in the street. The pick-up head extends across the entire width of the sweeper and uses rubber curtains on the front and back to create a seal on the road surface (Fleming 1978). The blast of air forces dirt and debris to become suspended (TYMCO 2012b). This debris-laden air is then sucked into the sweeper's hopper via a suction inlet. The larger air volume in the hopper slows the air, allowing heavier debris to fall. Lighter debris such as paper, plastic bags and leaves is trapped by a screen, while the lighter particulates are removed by a centrifugal dust separator (Fleming 1978). The cleaned air is then returned to the blower to restart the process. This closed-loop system prevents air from being exhausted to the atmosphere (Fleming 1978). Regenerative air sweepers are commonly grouped with vacuum sweepers (Brinkmann and Tobin 2001; USEPA 1985a). As with other sweeper types, gutter brooms may be used to direct dirt and debris to the path of a pick-up mechanism and a water spray mist may be used to suppress dust during the entire process (Sutherland 2011). Some pick-up heads may also contain a rotary brush to dislodge stuck-on dirt and debris (Elgin Sweeper 2012a; TYMCO 2012c).

Regenerative air sweepers are considered to be more environmentally friendly than mechanical and vacuum sweepers, because of their ability to sweep a wider path than vacuum sweepers, remove larger materials (viz., trash, road debris, and vegetation) and small and coarse particles found in cracks and uneven pavement, and to minimize the resuspension of particulate matter (Schilling 2005a; Sutherland 2011).

Uneven pavement may create fugitive dust losses from the pick-up head, but this loss is much less than from a typical vacuum sweeper (Sutherland 2011). Regenerative air sweepers generally do not remove wet vegetation or large road debris as well as mechanical sweepers. The requirement for pick-up head curtains to reinforce surface suction may cause larger materials to be pushed aside unless a pressure bleeder is used. Moreover, like all sweeper types, gutter brooms and the pick-up head broom may expose finer sized-particles for easy transport in stormwater (Schilling 2005a).

2.1.6 High-Efficiency Sweepers

High-efficiency sweepers are represented by any of the three main types (mechanical, vacuum, or regenerative air), provided they are modified to control the loss of fugitive dust smaller than 60 μm with the use of media particulate filters (Sutherland 2011). Most high-efficiency sweepers have the ability to suppress dust without using water (Sutherland 2011).

Sutherland credits himself for coining the term "high-efficiency" in 1997, when describing what he thought was the first sweeper to use a filtration system, and which would only exhaust particles with diameters less than 2.5 μm (Sutherland 2011). The device he described as being "high efficiency" was a vacuum sweeper developed in 1995 by EnviroWhirl Technologies in Centralia, IL, which was acquired by Schwarze Industries in 1999, and whose technology was then used in their EV-series, i.e., in their EV1 and EV2 models (Sutherland 2011).

In a computerized simulation comparing the EnviroWhirl sweeper with an Elgin Crosswind (regenerative air), and 1988 Mobil (mechanical), the EnviroWhirl reduced mean total suspended solids (TSS) by more than 80 %, followed by about 70 % for the Crosswind, and 20–30 % for the Mobil (Sutherland and Jelen 1997). Though the EV-series performed well in the simulation and was useful for waterless applications such as hazardous material cleanup, its high cost and limited maximum non-sweeping speed of 25 mph hindered its acceptance by the municipal market. The EV-series has since been discontinued (Sutherland 2011).

Several sweepers in the late 1970s were fitted with filters in an effort to control fugitive dust emissions; the filtration units on these had technical issues which led to loss of suction. Beginning in the early 1980s, the TYMCO Model 600DC (the predecessor to the DST-6), used media filters inside its hopper to control $PM_{2.5}$ emissions (TYMCO Inc. 2011, personal communication). TYMCO's DST-6 and DST-4 models clean all of the air by employing a centrifugal dust separator. After passing through the centrifugal dust separator, a small percentage of the air is exhausted to atmosphere after it is diverted to an external module containing filter cartridges, which are designed to remove particles as small as 0.5 μm. This causes an increase in suction around the pick-up head, thereby reducing fugitive emissions (Sutherland 2011; TYMCO 2012c).

Elgin Sweeper Company has three models that may be called "high-efficiency" sweepers: the Waterless Eagle and Waterless Pelican (mechanical) and the Crosswind NX (a regenerative air sweeper, which is no longer listed on Elgin's website) (Elgin Sweeper 2012b; Sutherland 2011). The waterless mechanical sweepers use a vacuum fan to siphon dust out of the hopper, shrouded gutter brooms, and a shrouded rotary broom. A filter in front of the fan traps dust and the cleaned air is exhausted (Sutherland 2011). The Crosswind NX is similar to the TYMCO DST-6 in that it siphons air from the hopper to an outside container. Rather than filter cartridges, the Crosswind NX use a series of filter bags, which purportedly remove over 99 % of particles as small as 0.5 μm (NAS 2012).

Schwarze Industries also has a high efficiency waterless model called the DXR, which uses a series of filter cartridges within the hopper, channeling 100 % of the air flow through a cartridge filter before it enters the blower. The DXR also employs shrouded gutter brooms with suction tubes to capture fugitive dust (Sutherland 2011). The DXR is not currently featured on Schwarze's website, and may have been discontinued.

2.2 Street Cleaning Purposes and Strategy

A survey of several municipalities across the United States suggests that street sweeping policy is dictated more by cleanliness and aesthetics than by potential water quality impacts. In communities with populations greater than 250,000, 11 %

were concerned about stormwater quality, 36 % were concerned about cleanliness, and 36 % were concerned about both categories (Brinkmann and Tobin 2001). Furthermore, the same survey disclosed that few cities have done research to assess their street sweeping practices or the effectiveness of their street cleaning program. Only 7 % of communities with populations of 5,000–25,000, 17 % of communities with populations of 25,000–100,000, and 24 % communities with populations >250,000 had done so (Brinkmann and Tobin 2001).

Several cities have implemented changes in street cleaning technologies as part of environmental management efforts. For example, the City of San Angelo, TX has implemented several changes to its street sweeping program to improve water quality of the North Concho River, including the use of 5 TYMCO Model 600 regenerative air sweepers, and using geospatial technology to record sweeping times and frequency. These changes resulted in pickup of 400–450 tons of material per year vs. the pre-change amounts of 200–250 tons of material per year (Talend 2012). Similarly, the City of Tacoma, Washington replaced its fleet of mechanical sweepers with 4 TYMCO Model 500x regenerative air sweepers and now uses geospatial technology to track sweeping implementation and to mark catch basins that require cleaning; this new strategy reduced the solids entering Commencement Bay via the Foss Waterway by more than half (Talend 2012). The City of Hamilton, Ontario, Canada and cooperative industry groups reduced ambient PM_{10} from 114 to 73 μg/m^3 by implementing several control measures, including street sweeping with TYMCO Model DST-6 high-efficiency regenerative air sweepers (DeLuca et al. 2012).

The California Stormwater Quality Association lists several suggested protocols for street sweeping and cleaning (CSQA 2003). These protocols include sweeping monthly at a minimum, sweeping in dry weather, avoiding flushing, increasing sweeping frequency in high traffic areas before the wet season, in special problem areas, at special events and in high litter zones, maintaining equipment in good order, and replacing older technologies with newer ones, preferably regenerative air (CSQA 2003).

A simulated study by the U.S. Geological Survey (USGS) suggested that solids and lead removal efficiency noticeably increased at sweeping frequencies of <7 days (Zarriello et al. 2002). A Florida study suggested that the optimum sweeping frequency for reducing street sediment for mechanical sweepers is once per week, the optimum frequency for reducing constituent loading in stormwater runoff is twice per week, and maintaining a frequent sweeping schedule is more important than storm intensity and duration in reducing sediment and pollutant loadings (Brinkmann and Tobin 2001). In contrast, a modeled USGS study of a commercial area indicated that sweeping once a month with a regenerative air sweeper is more effective than sweeping three times a week with mechanical or vacuum sweepers, judging by modeled reductions in the totals for solids, particulate solids, total phosphorus, and to particulate phosphorus (USGS 2013). Notwithstanding these commentaries, peer-reviewed empirical data supporting such management activities are not available.

2.3 Early Street Sweeper Studies

The earliest street sweeper studies were conducted by the U.S. Naval Radiological Defense Laboratory (NRDL) in the late 1950s and early 1960s to compare mechanical, vacuum, and flusher effectiveness at removing dry particulate matter (dry fallout material) from paved areas (Lee et al. 1959; Sartor and Boyd 1972). Sartor and Boyd (1972) reviewed the early studies by NRDL, along with other published data and information from street cleaning manufacturers, and performed an *in situ* evaluation of several U.S. cities, and conducted controlled tests using a simulated street surface contaminant. The NRDL data review showed that vacuum sweeping is more effective than mechanical sweeping for a "level of effort," as defined by the ratio of minutes of equipment operation to the surface area swept. The *in situ* evaluation indicated removal efficiencies ranging from 11 to 62 % for various mechanical sweepers, and the controlled study showed removal efficiencies of 26.5–77.7 % for a Mobil-TE-3 mechanical sweeper, and 36.0 and 44.2 % for a TYMCO Model 300 regenerative air sweeper (Sartor and Boyd 1972).

Axetell and Zell (1977) evaluated measures to control particulate air quality for the EPA. The capacity of mechanical, vacuum, and regenerative air sweepers (with and without flushing) to prevent re-entrainment of particulate matter was tested in a commercial area of Kansas City, Missouri and a residential area of Cincinnati, Ohio. After evaluating flushing and mechanical sweeping in Kansas City, flushing was found to be most effective at controlling air particulates, achieving a reduction of air particulate concentrations of 8–18 μg/m^3 after adjusting for differences in local air quality. The investigators reported that particulate concentrations were higher on the day of flushing and sweeping, but were lower than average on the next few days thereafter. In Cincinnati, flushing, mechanical sweeping, and vacuum sweeping were evaluated with contradictory results. Mechanical sweeping was considered to be the most effective for controlling particulate resuspension, with concentrations 6–20 μg/m^3 less than the other methods tested. Flushing showed no significant reduction, though concentrations were 16 μg/m^3 lower on the days that flushing was practiced and 4 μg/m^3 lower on the day after flushing. Vacuum sweeping was found to be ineffective, with concentrations increasing by 5 μg/m^3 when compared to no street cleaning (Axetell and Zell 1977). A vacuum sweeper was also evaluated in a suburb of Kansas City, and appeared to be effective at removing material from the street surface. However, study results showed no significant difference in air particulate levels compared to a nearby area that was not swept. This outcome may have resulted from low traffic density at the test site (Axetell and Zell 1977).

EPA established the Nationwide Urban Runoff Plan (NURP) in 1978. The program included a five year study designed to quantify the characteristics of urban runoff in different locations to determine the following: differences and similarities, how much urban runoff contributes to water quality problems across the nation, and the effectiveness of management practices for controlling pollutants in urban runoff. Study results showed street sweeping to be largely ineffective, with constituent reductions never exceeding 50 % in event mean concentrations (USEPA 1983).

Robert Pitt is credited with being the first to evaluate the effectiveness of street cleaning to manage storm water runoff by performing monitoring activities in an EPA funded study in San Jose, CA (Sutherland 2011; USEPA 1979). Pitt concluded that street sweepers were more effective at picking up larger size particles than smaller ones, that smaller size particles tended to increase over time, and pollutant concentrations tended to increase with decreasing particle size (USEPA 1979). Pitt also developed sampling procedures for evaluating street cleaning equipment under real-world conditions. The sampling technique utilized an industrial vacuum cleaner with a stainless steel canister. Street dirt was vacuumed along a randomly selected test strip within a test area from the curb to the center of the street before and after sweeping (USEPA 1979). This sampling technique is popular amongst researchers (USGS 2007; Law et al. 2008; DiBlasi 2008), because of its random nature and ease, but drawbacks that have been pointed out by Sutherland (2011) include possible street dirt accumulation if sampling is not done immediately after street sweeping, and parked cars present at the time of sweeping may not be present at the time of sampling and therefore may inhibit the accuracy of the measurements.

EPA tested the performance of a modified TYMCO Model 600 regenerative air sweeper, a standard TYMCO Model 600 regenerative air sweeper, and a Mobil mechanical sweeper. Modifications of the TYMCO Model 600 included partial hoods over the gutter brooms, venting the hoods to the hopper, and venting air out of the regenerative air system to increase suction with a low pressure drop venturi scrubber for dust suppression. Results of the study showed the modified TYMCO Model 600 was able to remove 80 % of solids, followed by 70 % and 20 % for the standard Model 600 and Mobil sweepers, respectively (USEPA 1985a).

2.4 Recent Sweeper Technology Comparison Studies

Though limited peer-reviewed information exists for comparisons of the effectiveness of sweeping methods, several researchers have attempted to compare effectiveness of different sweeper technologies. Evaluating sweeper efficiencies for street dust is difficult, because within the general sweeper technology categories of mechanical, vacuum and regenerative air, different manufacturers' sweeper models include subtle design modifications. In addition to differences amongst manufacturers, parameters in experimental designs may vary, including testing conditions, sampled material (simulated or as found *in situ*), sweeping speed, human error (adherence with standardized protocols, practices, procedures), and other factors. Despite such influencing variables, which inherently introduce uncertainty in environmental assessment and management efforts, we examine these more recent studies below.

Sutherland and Jelen (1997) conducted a simulated model study, in which sweeper test data from EPA's NURP studies of the late 1970s and early 1980s which indicated that sweeping was $<50\%$ effective at reducing constituents in runoff, were compared to data developed in the 1990s (USEPA 1983). The sweeper models compared included a ~1978 Mobil (mechanical) sweeper from Pitt (USEPA 1985b), a 1988 Mobil sweeper, a 1988 Mobil and a TYMCO regenerative air sweeper generically labeled as a vacuum, in tandem from HDR (1993), an Elgin Crosswind (regenerative air) sweeper from a test performed in 1995, and an EnviroWhirl (high efficiency vacuum) tested in two separate studies by Sutherland and Jelen in 1995. A comparison of residual dust remaining on the street indicated that most residuals remained after using a NURP era mechanical sweeper, followed by a newer mechanical sweeper, then the 1988 Mobil and TYMCO sweepers in tandem, finally followed by the Elgin Crosswind, and the EnviroWhirl sweepers. Though the EnviroWhirl sweeper was predicted to leave no residual dust on the street for any of the particle size groups examined, its efficiencies were modeled at less than 100 % for all particle sizes; the authors note that the seeming inconsistency in these modeled results arises from predictions for higher quantities of loading beyond the expected baseline. However, 100 % efficiencies were reportedly achieved for the newer mechanical sweeper in the lower particle size ranges <63 – 250 μm, and for the regenerative air sweeper in the 250–2,000 μm particle size ranges (Sutherland and Jelen 1997).

In another simulated study, USGS tested for expected efficiencies of a mechanical sweeper, wet vacuum and regenerative air sweeper, a dry vacuum, and a "best available technology" sweeper. The "best available technology" sweeper was described as having produced the highest efficiencies found in the literature (Zarriello et al. 2002). When averaging the expected removal efficiencies for each sweeper for suspended solids, fecal coliform bacteria, total phosphorus, and total lead, the best available technology was expected to achieve an efficiency of 93 %, followed by 63 % for the dry vacuum, 29 % for the wet vacuum and regenerative air method, and 11 % for the mechanical sweeper (Zarriello et al. 2002). The Florida Department of Transportation evaluated a mechanical sweeper and a regenerative air sweeper, and sweeper effectiveness was determined by measuring material left on the street after the sweeper had passed (Tobin and Brinkmann 2002). Coarse material was defined as material collected after the sweeper had passed, and which was collected by using a whisk-broom and dust pan. Fine material was defined as material collected by a shop-vacuum after the course material was collected. Very fine material was defined as material collected from a sandbag dammed area after flushing. In this study, a mechanical sweeper was more efficient at sediment removal, especially course sediment, than was a regenerative air sweeper. The mechanical sweeper in this study showed an overall effectiveness of 95–98 %, far beyond what has been observed in other studies of similar technologies. The regenerative air sweeper was better at removing very fine sediment (Brinkmann et al. 1999). Two water samples were also collected from the curbside reservoir and one from the water delivery system to serve as a background control (Brinkmann et al. 1999). The test material applied was previously collected street sweepings representative to the area. Each sweeper was evaluated on three test strips on a closed street on a dry, calm day. Each test strip was 1.5 m from the curb, was 10 m long, and had 25 kg of material spread evenly to a depth of 0.5 ± 0.2 cm, and had a minimum width of 1.25 m (Brinkmann et al. 1999).

Among comparative studies in the grey literature, perhaps the most robust was performed by the City of Toronto using Environmental Technology Verification (ETV) Canada General Verification Protocol (ETV 2012a, Toronto 2008). This test protocol evaluated the sweepers' pick-up efficiencies and the sweepers' ability to minimize PM_{10} and $PM_{2.5}$ emissions while sweeping (Toronto 2008). ETV has now verified four high-efficiency sweepers under the City of Toronto's PM_{10} and $PM_{2.5}$ Street Sweeper Efficiency Test Protocol. These studies were conducted in an 80×11 m enclosed tent, in which two 2.75×30 m strips of calcium carbonate powder were distributed onto aged pavement with cracks and potholes. The powder particles had a mean diameter of 3 μm and total weight of about 270 kg. Water was not used for dust suppression by any of the sweepers tested (City of Toronto 2008). The first sweeper, certified in 2005, a TYMCO Model DST-6 (regenerative air) removed >90 % of the test material from the surface. PM_{10} and $PM_{2.5}$ air contamination concentrations were measured as being below the limit of detection (LOD) (ETV 2012a). Comparing testing conducted in 2005 and 2008 is difficult as the limits of detection for PM were lowered in the second trial. Therefore, we will only compare the remaining three sweepers tested (ETV 2011, 2012a, b, c) below.

Of the tests performed in 2008, the Elgin Waterless Eagle and TYMCO Model DST-4 showed similar removal efficiencies of 88 % and 89 %, respectively, followed by the Elgin Crosswind NX with 82 % pick-up efficiency. Total PM_{10} air contamination concentrations were 11, 2.63, and 6.12 mg/m^3-kg for the DST-4, Waterless Eagle, and Crosswind NX, respectively. Total $PM_{2.5}$ air contamination concentrations were 7.5, 1.44, and 4.71 mg/m^3-kg for the DST-4, Waterless Eagle, and Crosswind NX, respectively (ETV 2011, 2012a, b, c). The only mechanical sweeper (the Waterless Eagle) controlled ambient PM better than the Crosswind NX, followed by the DST-4 (ETV 2011, 2012a, b, c). This performance by the Waterless Eagle may be attributed to its use of shrouded gutter brooms that enhanced vacuum suction, a feature not present on the other sweeper models tested. It should be noted that gutter broom shrouds were fastened in an elevated position that allowed the gutter brooms to make full contact with the curb during the test (ETV 2012c). Although shrouded gutter brooms with vacuum suction may be efficient in controlling ambient PM, it is also true that they may push larger debris such as leaves to create a bulldozing effect that prevents sweeping.

The USGS tested an Elgin Pelican Series P (mechanical sweeper) against a Johnston 605 (vacuum sweeper; USGS 2005). Street-sweeper efficiencies ranged from about 20–31 % for the mechanical sweeper and from about 60–92 % for the vacuum sweeper for the particle-size range tested. Efficiencies for particle sizes 2 mm-250 μm were at least 1.5–5 times greater for the vacuum sweeper than for the mechanical sweeper. The vacuum sweeper was at least 1.6–10 times more efficient than the mechanical one for all particle-size ranges examined (USGS 2005).

The USGS performed another study, in which they compared an Elgin Pelican (mechanical sweeper), an Elgin Crosswind (regenerative air sweeper) and an Elgin Whirlwind (vacuum sweeper) (USGS 2007). A regenerative air sweeper was replaced by a vacuum sweeper during this study, because industry representatives considered it to be more effective. These regenerative air and vacuum sweepers had similar pickup efficiencies (i.e., 25 and 30 %) compared to an average of 5 % of street dust yield for the mechanical sweeper. Average reductions in basin street dust yield were compared among three sweepers. A regenerative air sweeper provided the highest reduction (76 %), followed by 63 % and 20 % for the vacuum and mechanical sweepers, respectively. The discrepancy between street dust yield reductions and pick-up efficiencies may be attributed to the abrasive nature of the gutter and rotary brooms that generate smaller particle-size loads. This added load from the brooms may also negate the stormwater quality benefits of street sweeping USGS 2007).

The National Water Research Institute (NWRI) in Canada compared a regenerative air sweeper, a mechanical sweeper and a high efficiency regenerative air sweeper (NWRI 2007). Although the study did not identify brands or models of sweepers, the sweepers pictured appear to be an Elgin Air Bear, which is referred to as "old-technology regenerative air" (ORA), an Allianz 4000, which is referred to as "conventional mechanical" (CM), and a TYMCO Model DST-6, which is referred to as "new-technology regenerative air" (NRA). The ORA sweeper and the CM sweeper were examined at 8–15 km/h in 2004, while the NRA sweeper was tested at 5–8 km/h in 2005. The ORA sweeper was tested in the northbound side of the roadway, the CM sweeper was tested in the southbound, and the NRA sweeper was tested in both (NWRI 2007).

The NRA sweeper was the only sweeper that removed a statistically significant mass of solids from the road surface (48 kg/curb km), and appeared to remove solids to a "background" level (approximately 40–60 kg/curb km), beyond which further removal appeared unlikely. The CM sweeper also removed approximately 40 kg/curb km (on average), but the difference between the swept and unswept streets was not statistically significant. Similarly, the ORA sweeper showed no statistically significant reduction in street dust. Both the CM sweeper and the NRA sweeper provided consistent reductions in the largest size range >2,000 μm in the northbound lanes (58 % and 88 % respectively). The NRA sweeper removed 73 % of the total mass of particles that were >2,000 μm in the southbound lanes, and the ORA sweeper was unable to pick up particles sized >2,000 μm. Only the NRA sweeper was able to significantly pick up particles in the 64–2,000 and <64 μm size ranges (62 % and 35 % removal efficiencies, respectively). The performance difference of the NRA sweeper in the northbound side compared to the southbound side may be attributable to a difference in street dirt accumulation on the surface (NWRI 2007).

Runoff studies using a sealed catch basin insert and dechlorinated tap water from a garden hose equipped with a gentle rain-like spray head, showed that mobilized solids were relatively unchanged by sweeping (NWRI 2007). The study authors also examined the effectiveness of these sweepers on levels of environmental contaminants. Both the ORA and CM sweepers showed slight reductions of solids. The NRA sweeper showed an increase of solids in the northbound side, and only minor reductions in the southbound side. The difference in these results between the 2004 and 2005 tests may be attributed to changes in the test procedure mentioned above, with the higher pressure and acidity dislodging more solids. None of the sweepers showed significant reduction in total metals. Whereas total

Table 2 Efficacy and efficiency of street sweeper technologies

Technology	% Efficiency	Sweeper model	Particle size	Reference
Mechanical				
	13	Elgin Pelican Series P	<63 μm	USGS (2005)
	9–40	Elgin Pelican Series P	63–2,000 μm	USGS (2005)
	20–31	Elgin Pelican Series P	Overall	USGS (2005)
	No significant change	Allianz 4000[a]	<64 μm	NWRI (2007)
	No significant change	Allianz 4000[a]	64–2,000 μm	NWRI (2007)
	55	Allianz 4000[a]	>2,000 μm	NWRI (2007)
	−41 to 46	Elgin Pelican	Overall	USGS (2007)
Vacuum				
	39–81	Johnston 605 Series	<63 μm	USGS (2005)
	31–93	Johnston 605 Series	63–2,000 μm	USGS (2005)
	60–92	Johnston 605 Series	Overall	USGS (2005)
	−2 to 52	Elgin Whirlwind	Overall	USGS (2007)
Regenerative air				
	No significant change	Elgin Air Bear[a]	Overall	NWRI (2007)
	3–51	Elgin Crosswind	Overall	USGS (2007)
High efficiency mechanical				
	88.1	Elgin Waterless Eagle	3 μm mean	ETV (2012c)
High efficiency regenerative air				
	No significant change-35	TYMCO Model DST-6[a]	<64 μm	NWRI (2007)
	No significant change-62	TYMCO Model DST-6[a]	64–2,000 μm	NWRI (2007)
	73–88	TYMCO Model DST-6[a]	>2,000 μm	NWRI (2007)
	90	TYMCO Model DST-6	3 μm mean	ETV (2012a)
	89	TYMCO Model DST-4	3 μm mean	ETV (2011)
	81.8	Elgin Crosswind NX	3 μm mean	ETV (2012b)
	35.7–98.3	TYMCO DST-6	Overall	USGS (2013)

[a] Indicates the sweeper manufacturers and models were not named in the literature; however we have attempted to identify them by their respective photos

zinc showed no change, dissolved zinc showed reductions of 46 % for the ORA sweeper and 56 % for the NRA sweeper. There were no significant changes for polycyclic aromatic hydrocarbons (PAHs) for any of the sweepers (NWRI 2007).

Weston Solutions, Inc. (2010) weighed street dirt material that was collected on sweeping routes for a Johnston 4000 (mechanical), an Elgin Whirlwind (vacuum), and a Schwarze A7000 (regenerative air) street cleaning units and found that the vacuum sweeper was able to pick up 14–45 % more material weight than the regenerative air, and 15–65 % more material weight then the mechanical sweeper. Pick-up efficiency could not be calculated, because the amount of material on the street was not measured before and after the sweeper pass.

Most recently the USGS tested a TYMCO Model DST-6 (high efficiency regenerative air sweeper) on multifamily residential and commercial land use streets in Cambridge, MA (USGS 2013). A computer model was used to determine estimated percent reduction of solids contributing to stormwater with a single pass of the Model DST-6 vs. a mechanical and a vacuum sweeper with previously collected data from other studies at various sweeping frequencies. The model showed reductions of 2.7, 5.2, and 16 % for the mechanical, vacuum and regenerative air sweepers respectively with monthly sweeping. Bimonthy sweeping showed reductions of 3.3, 7.0, and 18 % for the mechanical, vacuum and regenerative air sweepers respectively. Weekly sweeping showed reductions of 4.2, 9.6, and 18 % for the mechanical, vacuum and regenerative air sweepers respectively. Sweeping three times per week showed reductions of 6.0, 14, 19 % for the mechanical, vacuum and regenerative air sweepers respectively. Although there was little improvement with increased sweeping frequency for the Model DST-6, sweeping monthly with it was still more effective than sweeping three times a week with mechanical or vacuum technologies (USGS 2013).

There are a limited number of studies, in which sweeper technologies were compared under well-defined experimental conditions. Differences in numerous study parameters (e.g., local land-uses, local geology and material studied, operational details, etc.), make it difficult to draw defensible conclusions from the literature about which technique or equipment type is optimum for various scenarios. Nevertheless, below, we have attempted to combine efficacy data from various studies to gain a better understanding of technology efficacy differences (Table 2).

Because simulated computer models were used to determine expected efficiencies, the papers by Sutherland and Jelen (1997), USGS (2013) and Zarriello et al. (2002) were excluded. The work of Brinkmann et al. (1999) was also excluded from our analysis, because their study only considered material left behind, which excluded material displaced outside of the sweeper and street. Another deficiency of this latter study is that particle size was not quantifiably defined. Weston Solutions (2010) was also excluded because pick-up efficiency could not be calculated. As expected, efficiencies were much higher for high efficiency sweepers, with an overall efficiency of 82 % for the Elgin Crosswind NX (regenerative air), 88 % for the Elgin Waterless Eagle (mechanical), 89 % for the TYMCO Model DST-4 (regenerative air), and 90 % for the TYMCO Model DST-6 (regenerative air) (ETV 2011, 2012a, b, c).

Table 2 highlights the high degree of variability amongst sweeper experiments performed to date, even when the same sweeper model was tested. For example, the TYMCO Model DST-6 was 90 % efficient in the ETV study, but only up to 35 %

efficient in the test performed by NWRI in the smallest particle size range (<63 µm) (ETV 2012a; NWRI 2007). Efficiency ranged from 36 to 98 % in the study conducted by USGS, which included 72 data points from commercial and residential neighborhoods (USGS 2013). There are several notable differences between these two studies; most notably that the ETV test used a test material with an average diameter of 3 µm in a controlled environment, while NWRI was a "real world" study (material of variable diameter from a non-controlled environment) (NWRI 2007; Rochfort 2012a). Similarly, the Elgin Pelican was up to 31 % efficient in the 2005 USGS test, while in the 2007 USGS test efficiencies ranged widely with the Pelican actually adding to street dirt yield by up to 41 % and reducing the street dirt yield by up to 46 % (Breault et al. 2005; Selbig and Bannerman 2007; Table 2). Interestingly, the vacuum sweeper performed better than the regenerative air sweeper as documented in various studies within the technology categories; however, again, there was a high degree of variability within and between studies, with overall removal efficiencies ranging from 60 to 92 % and -2 to 52 % in two separate USGS studies in which different vacuum sweeper models were evaluated and efficiencies ranging from no change and -3 to 51 % in studies by NWRI and USGS in which two different regenerative air sweeper models were evaluated (USGS 2005; NWRI 2007; Table 2) (USGS 2007).

3 Environmental Regulation in the US

The first major law to address water pollution in the US was the Federal Water Pollution Control Act of 1948 (FWPCA). Eventually, FWPCA was amended by the Clean Water Act (CWA) of 1972 to address public concerns associated with water pollution. The CWA outlined a regulatory structure for pollutant discharges to US waters, set standards for industry discharges and surface water quality, required a permit for point source discharges, established grants for municipal sewage treatment, and identified a planning need for the problems associated with nonpoint source pollution, including that from street surface runoff (USEPA 2013a). Of particular relevance to street cleaning is that point source and nonpoint source stormwater pollution are regulated by the National Pollutant Discharge Elimination System (NPDES) program for municipal separate storm sewer systems (MS4s), construction activities, and industrial activities by permit (USEPA 2013b).

NPDES permits for MS4s fall under two categories: phase I and phase II. Phase I, issued in 1990, requires stormwater discharge permits for medium and large cities, and some counties with populations of 100,000 or greater. Phase II, issued in 1999, requires stormwater discharge permits for small MS4s in and around urbanized areas (USEPA 2013c). NPDES requires six MS4 program elements called "minimum control measures" as follows: (1) public education and outreach (e.g., distribution of educational materials about the impacts of stormwater discharge on water quality); (2) public participation and involvement (e.g., public hearings on stormwater regulations); (3) detection and elimination of illicit discharges; (4) runoff control for construction sites; (5) pollution prevention/good housekeeping with control measures (e.g., regular street sweeping, and; (6) development and maintenance of on-site wastewater treatment systems (USEPA 2005a). To obtain a permit, MS4s operators must submit a Notice of Intent (NOI) including its chosen Best Management Practices (BMPs) and measureable goals for each minimum control measure (USEPA 2005a). BMPs include municipal landscaping, parking lot and street cleaning, storm drain system cleaning, and others (USEPA 2005b).

Another relevant street-cleaning regulation in the US was the Clean Air Act (CAA) of 1970 (USEPA 2013d). The CAA regulates emissions from stationary sources, such as coal fired power plants, and mobile sources, such as vehicles. Under the CAA, EPA sets National Ambient Air Quality Standards (NAAQS) to regulate hazardous emissions for the sake of public health and welfare (USEPA 2013d).

Arguably the NAAQS region most impacted by poor air quality is the South Coast Air Quality Management District (AQMD) covering all of Orange County, California, and the urban portions of Los Angeles, Riverside, and San Bernardino counties in Southern California. In an effort to reduce ambient PM loads from paved and unpaved roads and livestock operations, AQMD adopted Rule 1186 in 1997 (AQMD 2008). Regarding street sweepers, Rule 1186 requires municipal use street sweepers to be certified by the AQMD. Sweepers are required to have a minimum pick-up efficiency of the test material of 80 % (AQMD 2009). Though the original intent of the Rule 1186 sweeper test was to determine which sweepers were efficient at picking up PM_{10}, it has been criticized because the testing procedure never actually measures the particle size of the material removed (Sutherland 2011). In addition, only about 3 % of the test material had diameters of 10 μm or less; hence, with a minimum pick-up efficiency of 80 % a sweeper could easily be certified as compliant without picking up any PM_{10} material (Sutherland 2011).

4 Characteristics of Street Dust

Typical street sweepings consist of soil, sediment, small pieces of pavement, leaves and organic debris, and anthropogenic trash. Several factors may affect variability in the composition of street sweepings, including sweeping method and technology, street surface type, traffic load, geographic area, and weather (Jang et al. 2009). Dust or sediments found in street sweepings typically consist of local crustal material such as eroded rock and soil and anthropogenic materials such as eroded material from bricks, concrete, other building materials, construction trackout, and roadway debris (maintenance/traction sand, automotive debris, exhaust particles, asphalt, sealants, etc.) (Brinkmann and Tobin 2001). Previous studies have indicated that street dust accumulates fairly quickly after cleaning (9 lb per curb mile per day for the first four days after cleaning, and 2 lb per curb mile per day between four and nine days after cleaning), but rates of accumulation can vary widely from location to location (USEPA 1979; WDOT 2009). Seventy-five percent of the mass of street dust lies within 3 ft of the curb, and approximately 90 % within 8 ft (USGS 2007; USEPA 1985a).

Crustal material grain size naturally varies by location, resulting in unique site-specific conditions. For example, soil in Milwaukee, Wisconsin (and much of the northern Midwest to the northeast) tends to contain more clay from glacial tills, whereas materials in Sarasota, Florida contains more sand (Brinkmann and Tobin 2001). Anthropogenic sediment sources are not as prevalent on roadways as natural sources and tend to be coarser in nature (Brinkmann and Tobin 2001).

Particle size is particularly important, because pollutants tend to bind to smaller size particles more than to larger size particles, due to the negative electrical charge of smaller particles and the positive electrical charge of pollutants, and because smaller particles have a higher surface area (Brinkmann and Tobin 2001; Lau and Stenstrom 2005; Liebens 2001). Smaller size particles also have a low density, allowing them to be easily carried by surface runoff (Zhao et al. 2009b).

Herngren et al. (2006) analyzed road-deposited sediments for three different land uses (residential, industrial, commercial) in Queensland, Australia. The highest percentage of total mass collected was consistently in the 0.45–75 μm size range, followed by the 76–150 μm size range. It was found that >90 % of the particles at each site were below 150 μm. In Santa Monica, CA, Lau and Stenstrom (2005) collected street dust samples from 18 locations spanning 5 different land use patterns: industrial, roads (i.e., major intersections), multi-family residential, single family residential, and commercial areas. For combined land uses, 47.7 % of the total mass of street dust collected was in the 100–250 μm size range. In all of the land use areas, the 100–250 μm size range was considered to be the most important, with the exception of the single family residential areas, which showed a larger proportion of mass within the <43 μm size range. However, in Wisconsin it was observed that 75 % of particle mass was in size fractions greater than 250 μm, and that the sizes class smaller than 63 μm contained less than 5 % of the total mass. The variability in granulometry among these different sites is significant; many local factors are expected to contribute to this variability, and as the available technologies for street sweeping have been shown to be most efficacious for certain size classes, a scientific approach to street sweeping must include a characterization of site-specific granulometry.

4.1 Sources of Contaminants in Street Sweepings

Sources of contaminants in street sweepings and roadside sediment include: emissions from vehicles, tire wear components, road wear components, tire control additives, pesticides, fertilizers, and industrial emissions (Jang et al. 2009). From these sources, a number of chemical contaminants arise, including many heavy metals and organic compounds. For instance, manganese and chromium are associated predominantly with brake dust (Tandon et al. 2008), whereas sources of zinc include: exhaust emissions, tire and body wear of vehicles, fluid leakage from vehicles, galvanized steels in road structures, and the weathering of asphalt and concrete (Jang et al. 2009; Lindgren 1996). Sources of nickel include gasoline, oil, asphalt vehicle exhaust, and the weathering of asphalt and concrete (Lindgren 1996; Muschack 1990). Sources of copper include brake linings, tires, alloys in motor vehicles, and the weathering of asphalt and concrete (Jang et al. 2009; Sadiq et al. 1989). Aluminum, potassium, silica, calcium, titanium, and strontium are thought to arise in street dust from local crustal materials (i.e., soil, sand, etc.) (Abu-Allaban et al. 2003; Amato et al. 2010a, b). Organic contaminants, such as PAHs, phthalates, dioxins, furans, and pesticides, are frequently found in street dust. Sources of organics in street dust are obviously numerous, but arise from asphalt, motor oil, gasoline, tire particles, wood soot, and vegetation (USGS 2005). Nutrients are another contaminant of potential concern in street dust. Nutrients in street dust originate from lawns, pet wastes, failing septic systems, and atmospheric deposition from industry and automobile emissions (USEPA 2005c).

In the following sections, we more closely examine available information for these various contaminants in street dust. For example, the USGS examined accumulation rates and chemical composition of street dust in residential areas (USGS 2005). Substantial concentrations of trace metals and PAHs were found in residential areas, with several metals exceeding probable effects concentrations (PEC) for producing adverse biological effects to aquatic organisms within several particle size ranges, and two PAHs exceeding their exposure-based guidelines. The largest mass of heavy metals, including cadmium, chromium, copper, lead, nickel, and zinc made up about 30 % of total mass in the 250–2,000 μm size range, while the sum of parent PAHs made up about 27 %. The largest concentrations of heavy metals and PAHs were found in the smaller particle size range (<63 μm). PAH levels were similar to those measured by the USGS in asphalt and in used motor oil in an unpublished study (USGS 2005).

4.2 Metals

Metals that are prominent in street dust include antimony, arsenic, beryllium, bismuth, cadmium, cobalt, copper, gold, lead, mercury, nickel, palladium, selenium, silver, tellurium, thallium, tin, and zinc (Brinkmann and Tobin 2001; Table 3). In the studies we analyzed, aluminum, cadmium, chromium, copper, lead, nickel, and zinc were quantified most frequently. The geographic locations of these studies spanned Massachusetts and Florida, USA; Xincheng, China; Brisbane, Australia; and Jonkoping and Lulea, Sweden. No trends in amounts found were established based on geography, because the available data are not extensive enough to allow for a systematic evaluation.

In a few studies, the concentrations of contaminants found in street dust were compared to either background levels, soil cleanup target levels, probable effect concentrations or other benchmarks. In Florida, zinc and copper concentrations in street dust were found to be statistically greater ($p < 0.05$) that those found in

Table 3 A summary of metal levels detected in street dust samples

Metal	Concentration (ppm)	Particle fraction (μm)	Geographic location	Sample	Citation
Aluminum					
	7,600	125–250	New Bedford, MA, USA	Residential	USGS (2005)
	9,800	63–125	New Bedford, MA, USA	Residential	USGS (2005)
	18,000	<63	New Bedford, MA, USA	Residential	USGS (2005)
	0.01	<0.45	Brisbane, Australia	Residential	Herngren et al. (2006)
	0.01	<0.45	Brisbane, Australia	Industrial	Herngren et al. (2006)
	0.07	<0.45	Brisbane, Australia	Commercial	Herngren et al. (2006)
	8.80	0.45–75	Brisbane, Australia	Residential	Herngren et al. (2006)
	13.59	0.45–75	Brisbane, Australia	Industrial	Herngren et al. (2006)
	5.51	0.45–75	Brisbane, Australia	Commercial	Herngren et al. (2006)
	8.80	75–150	Brisbane, Australia	Residential	Herngren et al. (2006)
	0.63	75–150	Brisbane, Australia	Industrial	Herngren et al. (2006)
	3.52	75–150	Brisbane, Australia	Commercial	Herngren et al. (2006)
Antimony					
	6	125–250	New Bedford, MA, USA	Residential	USGS (2005)
	6	<63	New Bedford, MA, USA	Residential	USGS (2005)
Arsenic					
	6	125–250	New Bedford, MA, USA	Residential	USGS (2005)
	5	63–125	New Bedford, MA, USA	Residential	USGS (2005)
	9	<63	New Bedford, MA, USA	Residential	USGS (2005)
Barium					
	98	125–250	New Bedford, MA, USA	Residential	USGS (2005)
	110	63–125	New Bedford, MA, USA	Residential	USGS (2005)
	210	<63	New Bedford, MA, USA	Residential	USGS (2005)

(continued)

Table 3 (continued)

Metal	Concentration (ppm)	Particle fraction (μm)	Geographic location	Sample	Citation
Beryllium					
	0.5	125–250	New Bedford, MA, USA	Residential	USGS (2005)
	0.6	63–125	New Bedford, MA, USA	Residential	USGS (2005)
	0.9	<63	New Bedford, MA, USA	Residential	USGS (2005)
Cadmium					
	0.5–1.5	<500	Bordeaux, France	Bridges and highway	Durand et al. (2003)
	1	125–250	New Bedford, MA, USA	Residential	USGS (2005)
	2	63–125	New Bedford, MA, USA	Residential	USGS (2005)
	3	<63	New Bedford, MA, USA	Residential	USGS (2005)
	0.003	<0.45	Brisbane, Australia	Commercial	Herngren et al. (2006)
	0.002	0.45–75	Brisbane, Australia	Residential	Herngren et al. (2006)
	0.002	0.45–75	Brisbane, Australia	Commercial	Herngren et al. (2006)
	0.002	75–150	Brisbane, Australia	Residential	Herngren et al. (2006)
	0.01	75–150	Brisbane, Australia	Commercial	Herngren et al. (2006)
Chromium					
	33–70	<500	Bordeaux, France	Bridges and highway	Durand et al. (2003)
	350	125–250	New Bedford, MA, USA	Residential	USGS (2005)
	300	63–125	New Bedford, MA, USA	Residential	USGS (2005)
	200	<63	New Bedford, MA, USA	Residential	USGS (2005)
	193	<63	Xincheng, China	Street Dust	Zhao et al. (2009a)
	168	125–63	Xincheng, China	Street Dust	Zhao et al. (2009a)
	133	250–125	Xincheng, China	Street Dust	Zhao et al. (2009a)

(continued)

Table 3 (continued)

Metal	Concentration (ppm)	Particle fraction (μm)	Geographic location	Sample	Citation
	0.02	0.45–75	Brisbane, Australia	Residential	Herngren et al. (2006)
	0.06	0.45–75	Brisbane, Australia	Industrial	Herngren et al. (2006)
	0.03	0.45–75	Brisbane, Australia	Commercial	Herngren et al. (2006)
	0.01	75–150	Brisbane, Australia	Residential	Herngren et al. (2006)
	0.003	75–150	Brisbane, Australia	Industrial	Herngren et al. (2006)
	0.02	75–150	Brisbane, Australia	Commercial	Herngren et al. (2006)
	0.01	151–300	Brisbane, Australia	Residential	Herngren et al. (2006)
	0.002	151–300	Brisbane, Australia	Industrial	Herngren et al. (2006)
	0.01	151–300	Brisbane, Australia	Commercial	Herngren et al. (2006)
Cobalt					
	6	125–250	New Bedford, MA, USA	Residential	USGS (2005)
	9	63–125	New Bedford, MA, USA	Residential	USGS (2005)
	11	<63	New Bedford, MA, USA	Residential	USGS (2005)
Copper					
	67–151	<500	Bordeaux, France	Bridges and highway	Durand et al. (2003)
	91	125–250	New Bedford, MA, USA	Residential	USGS (2005)
	140	63–125	New Bedford, MA, USA	Residential	USGS (2005)
	250	<63	New Bedford, MA, USA	Residential	USGS (2005)
	285	<63	Xincheng, China	Street Dust	Zhao et al. (2009a)
	258	125–63	Xincheng, China	Street Dust	Zhao et al. (2009a)
	182	250–125	Xincheng, China	Street Dust	Zhao et al. (2009a)
	0.08	<0.45	Brisbane, Australia	Residential	Herngren et al. (2006)
	0.01	<0.45	Brisbane, Australia	Industrial	Herngren et al. (2006)

(continued)

Table 3 (continued)

Metal	Concentration (ppm)	Particle fraction (μm)	Geographic location	Sample	Citation
	0.12	<0.45	Brisbane, Australia	Commercial	Herngren et al. (2006)
	0.56	0.45–75	Brisbane, Australia	Residential	Herngren et al. (2006)
	0.96	0.45–75	Brisbane, Australia	Industrial	Herngren et al. (2006)
	0.26	0.45–75	Brisbane, Australia	Commercial	Herngren et al. (2006)
	0.48	75–150	Brisbane, Australia	Residential	Herngren et al. (2006)
	0.04	75–150	Brisbane, Australia	Industrial	Herngren et al. (2006)
	0.24	75–150	Brisbane, Australia	Commercial	Herngren et al. (2006)
Iron					
	33,000	125–250	New Bedford, MA, USA	Residential	USGS (2005)
	34,000	63–125	New Bedford, MA, USA	Residential	USGS (2005)
	33,000	<63	New Bedford, MA, USA	Residential	USGS (2005)
	0.01	<0.45	Brisbane, Australia	Residential	Herngren et al. (2006)
	0.96	<0.45	Brisbane, Australia	Commercial	Herngren et al. (2006)
	14.00	0.45–75	Brisbane, Australia	Residential	Herngren et al. (2006)
	43.96	0.45–75	Brisbane, Australia	Industrial	Herngren et al. (2006)
	16.78	0.45–75	Brisbane, Australia	Commercial	Herngren et al. (2006)
	12.80	75–150	Brisbane, Australia	Residential	Herngren et al. (2006)
	2.16	75–150	Brisbane, Australia	Industrial	Herngren et al. (2006)
	9.59	75–150	Brisbane, Australia	Commercial	Herngren et al. (2006)
Lanthanum					
	12	125–250	New Bedford, MA, USA	Residential	USGS (2005)
	18	63–125	New Bedford, MA, USA	Residential	USGS (2005)
	24	<63	New Bedford, MA, USA	Residential	USGS (2005)

(continued)

Table 3 (continued)

Metal	Concentration (ppm)	Particle fraction (μm)	Geographic location	Sample	Citation
Lead					
	96–222	<500	Bordeaux, France	Bridges and highway	Durand et al. (2003)
	420	125–250	New Bedford, MA, USA	Residential	USGS (2005)
	490	63–125	New Bedford, MA, USA	Residential	USGS (2005)
	1,240	<63	New Bedford, MA, USA	Residential	USGS (2005)
	311	<63	Xincheng, China	Street Dust	Zhao et al. (2009a)
	333	125–63	Xincheng, China	Street Dust	Zhao et al. (2009a)
	203	250–125	Xincheng, China	Street Dust	Zhao et al. (2009a)
	0.01	<0.45	Brisbane, Australia	Commercial	Herngren et al. (2006)
	0.04	0.45–75	Brisbane, Australia	Residential	Herngren et al. (2006)
	0.96	0.45–75	Brisbane, Australia	Industrial	Herngren et al. (2006)
	0.34	0.45–75	Brisbane, Australia	Commercial	Herngren et al. (2006)
	0.03	75–150	Brisbane, Australia	Residential	Herngren et al. (2006)
	0.04	75–150	Brisbane, Australia	Industrial	Herngren et al. (2006)
	0.19	75–150	Brisbane, Australia	Commercial	Herngren et al. (2006)
Lithium					
	9	125–250	New Bedford, MA, USA	Residential	USGS (2005)
	12	63–125	New Bedford, MA, USA	Residential	USGS (2005)
	22	<63	New Bedford, MA, USA	Residential	USGS (2005)
Manganese					
	350	125–250	New Bedford, MA, USA	Residential	USGS (2005)
	400	63–125	New Bedford, MA, USA	Residential	USGS (2005)
	440	<63	New Bedford, MA, USA	Residential	USGS (2005)

(continued)

Table 3 (continued)

Metal	Concentration (ppm)	Particle fraction (μm)	Geographic location	Sample	Citation
	0.01	<0.45	Brisbane, Australia	Residential	Herngren et al. (2006)
	0.02	<0.45	Brisbane, Australia	Industrial	Herngren et al. (2006)
	0.27	<0.45	Brisbane, Australia	Commercial	Herngren et al. (2006)
	0.23	0.45–75	Brisbane, Australia	Residential	Herngren et al. (2006)
	0.51	0.45–75	Brisbane, Australia	Industrial	Herngren et al. (2006)
	0.22	0.45–75	Brisbane, Australia	Commercial	Herngren et al. (2006)
	0.20	75–150	Brisbane, Australia	Residential	Herngren et al. (2006)
	0.02	75–150	Brisbane, Australia	Industrial	Herngren et al. (2006)
	0.16	75–150	Brisbane, Australia	Commercial	Herngren et al. (2006)
Molybdenum					
	4	125–250	New Bedford, MA, USA	Residential	USGS (2005)
	4	63–125	New Bedford, MA, USA	Residential	USGS (2005)
	5	<63	New Bedford, MA, USA	Residential	USGS (2005)
Nickel					
	67–151	<500	Bordeaux, France	Bridges and highway	Durand et al. (2003)
	35	125–250	New Bedford, MA, USA	Residential	USGS (2005)
	44	63–125	New Bedford, MA, USA	Residential	USGS (2005)
	55	<63	New Bedford, MA, USA	Residential	USGS (2005)
	165	<63	Xincheng, China	Street Dust	Zhao et al. (2009a)
	135	125–63	Xincheng, China	Street Dust	Zhao et al. (2009a)
	96	250–125	Xincheng, China	Street Dust	Zhao et al. (2009a)

(continued)

Table 3 (continued)

Metal	Concentration (ppm)	Particle fraction (μm)	Geographic location	Sample	Citation
Scandium					
	2.7	125–250	New Bedford, MA, USA	Residential	USGS (2005)
	3.8	63–125	New Bedford, MA, USA	Residential	USGS (2005)
	3.9	<63	New Bedford, MA, USA	Residential	USGS (2005)
Silver					
	0.4	125–250	New Bedford, MA, USA	Residential	USGS (2005)
	1.0	63–125	New Bedford, MA, USA	Residential	USGS (2005)
	1.2	<63	New Bedford, MA, USA	Residential	USGS (2005)
Strontium					
	30	125–250	New Bedford, MA, USA	Residential	USGS (2005)
	36	63–125	New Bedford, MA, USA	Residential	USGS (2005)
	46	<63	New Bedford, MA, USA	Residential	USGS (2005)
Tin					
	12	63–125	New Bedford, MA, USA	Residential	USGS (2005)
	19	<63	New Bedford, MA, USA	Residential	USGS (2005)
Titanium					
	800	125–250	New Bedford, MA, USA	Residential	USGS (2005)
	100	63–125	New Bedford, MA, USA	Residential	USGS (2005)
	1,300	<63	New Bedford, MA, USA	Residential	USGS (2005)
Vanadium					
	36	125–250	New Bedford, MA, USA	Residential	USGS (2005)
	49	63–125	New Bedford, MA, USA	Residential	USGS (2005)
	75	<63	New Bedford, MA, USA	Residential	USGS (2005)
Yttrium					
	11	125–250	New Bedford, MA, USA	Residential	USGS (2005)
	13	63–125	New Bedford, MA, USA	Residential	USGS (2005)
	15	<63	New Bedford, MA, USA	Residential	USGS (2005)

(continued)

Table 3 (continued)

Metal	Concentration (ppm)	Particle fraction (μm)	Geographic location	Sample	Citation
Zinc					
	249–547	<500	Bordeaux, France	Bridges and highway	Durand et al. (2003)
	270	125–250	New Bedford, MA, USA	Residential	USGS (2005)
	320	63–125	New Bedford, MA, USA	Residential	USGS (2005)
	810	<63	New Bedford, MA, USA	Residential	USGS (2005)
	529	<63	Xincheng, China	Street Dust	Zhao et al. (2009a)
	438	125–63	Xincheng, China	Street Dust	Zhao et al. (2009a)
	384	250–125	Xincheng, China	Street Dust	Zhao et al. (2009a)
	0.39	<0.45	Brisbane, Australia	Residential	Herngren et al. (2006)
	0.18	<0.45	Brisbane, Australia	Industrial	Herngren et al. (2006)
	0.83	<0.45	Brisbane, Australia	Commercial	Herngren et al. (2006)
	1.80	0.45–75	Brisbane, Australia	Residential	Herngren et al. (2006)
	2.32	0.45–75	Brisbane, Australia	Industrial	Herngren et al. (2006)
	0.42	0.45–75	Brisbane, Australia	Commercial	Herngren et al. (2006)
	0.72	75–150	Brisbane, Australia	Residential	Herngren et al. (2006)
	0.11	75–150	Brisbane, Australia	Industrial	Herngren et al. (2006)
	0.30	75–150	Brisbane, Australia	Commercial	Herngren et al. (2006)
Zirconium					
	13	125–250	New Bedford, MA, USA	Residential	USGS (2005)
	10	63–125	New Bedford, MA, USA	Residential	USGS (2005)
	7.1	<63	New Bedford, MA, USA	Residential	USGS (2005)

Florida soils (Jang et al. 2009). USGS (2005) tested street dust for metals and found that beryllium and lead concentrations exceeded Massachusetts Department of Environmental Protection exposure-based soil standards by 1.3 and 4.1 times, respectively, in the smallest particle size range (<63 μm). Lead also exceeded exposure-based guidelines for all particle size ranges <250 μm, and its whole sample concentration was 1.1 times greater than its exposure-based guideline.

USGS (2005) compared metal concentrations to PECs for adverse biological effects in benthic organisms. Chromium and nickel exceeded their respective PECs in all particle size ranges except for those >2,000 μm. Cadmium and zinc were higher than their PECs only in the smallest particle-size range (<63 μm), whereas copper exceeded its PEC in the smallest and largest particle size ranges of <63 μm and >2,000 μm, respectively.

Zhao et al. (2009a) analyzed heavy metals (e.g., chromium, copper, nickel, lead, and zinc) in street dust particles in a small town near the Yangtze River delta in China and found that about 63–71 % of their mass resided in particles of the <250 μm size range, which accounted for 40 % of the total mass of material collected from the street. Among land use categories, industrial areas had the highest concentration of metals, followed by main traffic roads, old residential, commercial, and new residential roads, respectively.

In Queensland, Australia, Herngren et al. (2006) analyzed street dust from residential, industrial, and commercial areas for eight heavy metals, and found that the highest concentrations were consistently found in the 0.45–75 μm size range overall. More than 90 % of the total mass of street dust collected was smaller than 150 μm, and half of the total heavy metal concentrations were found in the <75 μm range. For all metals tested, the highest concentrations were found at the industrial site, although cadmium results were usually below the detection limit. The highest concentrations found were usually for iron and aluminum.

In each of the studies examined above, concentrations generally increased with decreasing particle size.

4.2.1 Aluminum

Aluminum in street dust primary arises from local soils (Amato et al. 2010b). The highest concentrations from the selected studies were recorded in New Bedford, Massachusetts where total aluminum concentrations ranged from 6,700 to 18,000 mg/kg, followed by Pensacola and Escambia County, Florida, where concentrations ranged from 1,278 to 17,312 mg/kg (Liebens 2001; USGS 2005). Concentrations in Brisbane, Australia were comparatively lower, ranging from 0.01 to 13.59 mg/kg (Herngren et al. 2006).

4.2.2 Cadmium

Cadmium in street dust arises from vehicle exhaust, tire wear, and industrial emissions (Hood 2006; Legret and Pagotto 1999). In the selected studies, concentrations were the highest (3 mg/kg) in the lowest particle size range (<63 μm) in Massachusetts (USGS 2005). Concentrations detected in other regions were comparatively lower, ranging from below the limit of detection to 1.37 mg/kg in Florida, below the detection limit to 0.01 mg/kg in Australia, and 0.049–0.23 mg/kg in Sweden (Herngren et al. 2006; Liebens 2001; Viklander 1998).

4.2.3 Chromium

Chromium is mainly associated with brake dust (Tandon et al. 2008). There is a high degree of variability in amounts of chromium detected in the various studies. For example, maximum concentrations were as high as 193 mg/kg (Xincheng, China) and 200 mg/kg (New Bedford, Massachusetts) in particles <63 μm, but, elsewhere, levels as low as 0.02–0.06 mg/kg (Brisbane, Australia) were observed for comparable size ranges (USGS 2005; Herngren et al. 2006; Zhao et al. 2009a). Concentrations tended to increase as particle size ranges decreased. Concentrations in China were much higher than in other countries, and ranged from 87 mg/kg for the largest particle size range (250–900 μm) to 193 mg/kg for the smallest particle size range (<63 μm) (Zhao et al. 2009a). Concentrations were lowest in Australia (Herngren et al. 2006) (Table 3). Of the two studies conducted in the United States, residential concentrations were as much as 35 times lower in Florida than in Massachusetts (Liebens 2001; USGS 2005).

4.2.4 Copper

Sources of copper include brake linings, tires, motor vehicle alloys, and weathered pavement (Jang et al. 2009; Sadiq et al. 1989). Levels at which copper is detected also has a high degree of site-specific variability. Concentrations of copper among the selected studies were highest in China and Massachusetts in the lowest particle size range (<63 μm) and in Sweden (German and Svensson 2002; USGS 2005; Zhao et al. 2009a). Concentrations were much lower in Brisbane in all particle size ranges (Herngren et al. 2006).

4.2.5 Lead

Traditional sources of lead are leaded paint and gasoline (Gulson et al. 1995). Although lead has been banned from paint and gasoline in the United States since 1978, lead may still come from washoff of lead paint from older buildings and structures (Davis et al. 2001). Maximum lead concentrations detected were 1,240 mg/kg in Massachusetts, with whole sample concentrations slightly exceeding the exposure-based guideline value (USGS 2005) (Table 3). The second highest lead concentration (311 mg/kg) was recorded in China at (Zhao et al. 2009a). Concentrations of lead were measured between 0.01 and 0.96 mg/kg (multiple particle size fractions) in eastern Australia, and orders of magnitude higher in the United States, ranging as high as 1,240 mg/kg in Massachusetts, and 94 mg/kg in Florida (Herngren et al. 2006; Jang et al. 2009; Liebens 2001; USGS 2005). Lead concentrations were comparable in two separate studies in Florida (18.3 mg/kg and 19.98 mg/kg) (Jang et al. 2009; Liebens 2001). Concentrations in Sweden were 45 mg/kg for particle sizes <0.25 mm (German and Svensson 2002) (Table 3).

4.2.6 Nickel

Nickel is associated with gasoline, oil, asphalt, vehicle exhaust, and the weathering of asphalt and concrete (Lindgren 1996; Muschack 1990). Nickel concentrations were greatest in China, followed by Massachusetts in the lowest particle size range (<63 μm) (USGS 2005; Zhao et al. 2009a). Nickel concentrations in Florida were lowest in two separate studies (Jang et al. 2009; Liebens 2001). Sweden concentrations were in the middle range (~25 mg/kg in the <0.25 mm range) (German and Svensson 2002).

4.2.7 Zinc

Zinc is associated with vehicle exhaust, tires, vehicle body wear, and fluid leakage from vehicles, weathered steel structures, and weathering of pavement (Jang et al. 2009; Lindgren 1996). Zinc concentrations were highest in Massachusetts in the lowest particle size range (<63 μm) (USGS 2005). In China, concentrations were comparatively lower in the <63 μm range (Zhao et al. 2009a). The lowest concentrations were found in Brisbane, Australia and these ranged from 0.04 to 2.32 in all particle size ranges (Herngren et al. 2006). In two separate studies performed in Florida, concentrations in the middle range were reported (viz., 65.1 and 38.48 mg/kg) without particle size being noted (Jang et al. 2009; Liebens 2001). Concentrations in Sweden were relatively higher at 257 ± 40 in the <0.25 mm particle size range (German and Svensson 2002).

4.3 Organic Contaminants

Although metals are the most frequently studied contaminants in street dust, PAH concentrations have been frequently reported in this medium and in stormwater runoff (Jang et al. 2009; NWRI 2007; USGS 2005; Zhao et al. 2008; Zhao et al. 2009a; Table 4). PAHs in street dust originate from vehicle exhaust, motor, waste oil and greases, gasoline, tire and asphalt particles, and wood soot (Jang et al. 2009; Takada et al. 1990; USGS 2005). A forensic study in urban Beijing, China found that vehicle emissions contributed to 57 % of PAHs in road dust, followed by 42 % contribution from coal/oil combustion (Zhang et al. 2008). In the United States and other Western countries, where coal is not used for residential heating, the profile of PAH sources would clearly be expected to differ. For example, a study conducted in Paris indicated that traffic was the primary contributor to PAHs there (Gasperi et al. 2005). Tire and brake lining particles contain noteworthy concentrations of a number of PAHs (Rogge et al. 1993). It has also been noted that vehicular PAHs may adsorb to road salt particles, which would suggest that colder weather environments may have higher concentrations of PAHs in spring runoff (Harrison et al. 1996). Furthermore, although traffic is a predominant source, industrial activities are also an important contributor (Hoffman et al. 1984). Numerous studies conducted by USGS have documented increasing environmental concentrations of PAHs attributable to the use of coal-tar based pavement sealants, though this has not been directly assessed in street dust to date (Van Metre et al. 2009; Mahler et al. 2010).

Lau and Stenstrom (2005) found that heavier molecular weight PAHs, such as chrysene and benz[a]anthracene, increased as particle size decreased, with concentrations as much as 10 times more in the <43 μm range than the 250–841 μm range in commercial and residential areas. This is consistent with findings reported elsewhere (Krein and Schorer 2000; Yang et al. 1997; Zhao et al. 2009b). However, no such trend was found in lower molecular weight PAHs such as biphenyl and acenaphthene (Krein and Schorer 2000; Lau and Stenstrom 2005).

Florida street sweepings were tested for 74 volatile organic compounds (VOCs) and 116 semi-volatile organic compounds (SVOCs) (Jang et al. 2009). Five VOCs were detected including: n-butyl benzene, isopropyl benzene, isopropyl toluene, 1,3,5-trimethylebenezne, and o-xylene in some samples. PAHs were also detected in a small number of the 169 samples analyzed (Jang et al. 2009). Some of the samples contained two base–neutral phthalate compounds, bis(2-ethylhexyl) phthalate and di-n-butyl phthalate, which are phthalic acid esters (PAE). The source of phthalates in street dust is likely discarded plastic materials (Jang et al. 2009).

USGS (2005) detected 27 out of 30 PAH analytes in street dust, with concentrations increasing with decreasing particle size. Four PAH concentrations were measured above their exposure-based guidelines of 0.7 mg/kg (MDEP 1996; method 2, soil category S-1), including benzo[*a*]anthracene and indeno[1,2,3-*cd*] pyrene concentrations (<63 μm) (Table 4). Benzo[*a*]pyrene concentrations also exceeded these guidelines in the 63–125 μm size range. Benzo[*b*]fluoranthene concentrations exceeded exposure-based guidelines by 1.2, 1.8, and 2.7 times, in the <63 μm, 63–125 μm, and 125–250 μm particle size ranges respectively.

Pesticides have also been detected in street dust. Low concentrations of DDT (25.1–461 mg/kg) were detected in 18 % of street sweeping samples in Florida, though it has not been in use for almost 40 years. In the same study, endosulfan II

Table 4 A summary of organic contaminant levels detected in street dust samples

Analyte	Concentration (ppm)	Particle fraction	Geographic location	Street type	Citation
Acenaphthene					
	0.033	<63 µm	Xincheng	Various	Zhao et al. (2009b)
	0.033	<63 µm	Xincheng	Various	Zhao et al. (2008)
	0.03	63–125 µm	Xincheng	Various	Zhao et al. (2008)
	0.029	125–250 µm	Xincheng	Various	Zhao et al. (2008)
	0.017	250–900 µm	Xincheng	Various	Zhao et al. (2008)
	0.033	<63 µm	New Bedford	Residential	USGS (2005)
	0.052	63–125 µm	New Bedford	Residential	USGS (2005)
	0.031	125–250 µm	New Bedford	Residential	USGS (2005)
	0.025	250–2,000 µm	New Bedford	Residential	USGS (2005)
Acenaphthylene					
	0.086	<63 µm	Xincheng	Various	Zhao et al. (2009b)
	0.086	<63 µm	Xincheng	Various	Zhao et al. (2008)
	0.083	63–125 µm	Xincheng	Various	Zhao et al. (2008)
	0.072	125–250 µm	Xincheng	Various	Zhao et al. (2008)
	0.043	250–900 µm	Xincheng	Various	Zhao et al. (2008)
	0.19	<63 µm	New Bedford	Residential	USGS (2005)
	0.076	63–125 µm	New Bedford	Residential	USGS (2005)
	0.045	125–250 µm	New Bedford	Residential	USGS (2005)
	0.037	250–2,000 µm	New Bedford	Residential	USGS (2005)
Anthracene					
	ND–680	<500 µm	Bordeaux	Bridges and highway	Durand et al. (2003)
	0.168	<63 µm	Xincheng	Various	Zhao et al. (2009b)
	0.168	<63 µm	Xincheng	Various	Zhao et al. (2008)
	0.172	63–125 µm	Xincheng	Various	Zhao et al. (2008)
	0.161	125–250 µm	Xincheng	Various	Zhao et al. (2008)
	0.113	250–900 µm	Xincheng	Various	Zhao et al. (2008)
	0.31	<63 µm	New Bedford	Residential	USGS (2005)

0.2	63–125 µm	New Bedford	Residential	USGS (2005)
0.12	125–250 µm	New Bedford	Residential	USGS (2005)
0.084	250–2,000 µm	New Bedford	Residential	USGS (2005)
Benzo(a)anthracene				
ND–1,500	<500 µm	Bordeaux	Bridges and highway	Durand et al. (2003)
0.015	Raw sweepings (<4.75 mm)	Florida	Various municipal	Jang et al. (2009)
0.364	<63 µm	Xincheng	Various	Zhao et al. (2009b)
0.364	<63 µm	Xincheng	Various	Zhao et al. (2009b)
0.283	63–125 µm	Xincheng	Various	Zhao et al. (2009b)
0.218	125–250 µm	Xincheng	Various	Zhao et al. (2009b)
0.167	250–900 µm	Xincheng	Various	Zhao et al. (2009b)
1.02	<63 µm	New Bedford	Residential	USGS (2005)
0.69	63–125 µm	New Bedford	Residential	USGS (2005)
0.45	125–250 µm	New Bedford	Residential	USGS (2005)
0.22	250–2,000 µm	New Bedford	Residential	USGS (2005)
Benzo(a)pyrene				
0.009	Raw sweepings (<4.75 mm)	Florida	Various municipal	Jang et al. (2009)
0.537	<63 µm	Xincheng	Various	Zhao et al. (2009b)
0.537	<63 µm	Xincheng	Various	Zhao et al. (2009b)
0.37	63–125 µm	Xincheng	Various	Zhao et al. (2009b)
0.246	125–250 µm	Xincheng	Various	Zhao et al. (2009b)
0.157	250–900 µm	Xincheng	Various	Zhao et al. (2009b)
1.4	<63 µm	New Bedford	Residential	USGS (2005)
0.96	63–125 µm	New Bedford	Residential	USGS (2005)
0.58	125–250 µm	New Bedford	Residential	USGS (2005)
0.25	250–2,000 µm	New Bedford	Residential	USGS (2005)
Benzo(e)pyrene				
1.4	<63 µm	New Bedford	Residential	USGS (2005)
0.76	63–125 µm	New Bedford	Residential	USGS (2005)
0.49	125–250 µm	New Bedford	Residential	USGS (2005)
0.22	250–2,000 µm	New Bedford	Residential	USGS (2005)

(continued)

Table 4 (continued)

Analyte	Concentration (ppm)	Particle fraction	Geographic location	Street type	Citation
Benzo(b)fluoranthene					
	ND–360	<500 µm	Bordeaux	Bridges and highway	Durand et al. (2003)
	0.0132	Raw sweepings (<4.75 mm)	Florida	Various municipal	Jang et al. (2009)
	0.917	<63 µm	Xincheng	Various	Zhao et al. (2009b)
	0.917	<63 µm	Xincheng	Various	Zhao et al. (2008)
	0.736	63–125 µm	Xincheng	Various	Zhao et al. (2008)
	0.531	125–250 µm	Xincheng	Various	Zhao et al. (2008)
	0.224	250–900 µm	Xincheng	Various	Zhao et al. (2008)
	1.87	<63 µm	New Bedford	Residential	USGS (2005)
	0.25	63–125 µm	New Bedford	Residential	USGS (2005)
	0.84	125–250 µm	New Bedford	Residential	USGS (2005)
	0.33	250–2,000 µm	New Bedford	Residential	USGS (2005)
Benzo(g,h,i)perylene					
	0.0076	Raw sweepings (<4.75 mm)	Florida	Various municipal	Jang et al. (2009)
	0.491	<63 µm	Xincheng	Various	Zhao et al. (2009b)
	0.491	<63 µm	Xincheng	Various	Zhao et al. (2008)
	0.377	63–125 µm	Xincheng	Various	Zhao et al. (2008)
	0.288	125–250 µm	Xincheng	Various	Zhao et al. (2008)
	0.164	250–900 µm	Xincheng	Various	Zhao et al. (2008)
	1.23	<63 µm	New Bedford	Residential	USGS (2005)
	0.716	63–125 µm	New Bedford	Residential	USGS (2005)
	0.39	125–250 µm	New Bedford	Residential	USGS (2005)
	0.13	250–2,000 µm	New Bedford	Residential	USGS (2005)
Benzo(k)fluoranthene					
	0.315	<63 µm	Xincheng	Various	Zhao et al. (2009b)
	0.315	<63 µm	Xincheng	Various	Zhao et al. (2008)
	0.227	63–125 µm	Xincheng	Various	Zhao et al. (2008)
	0.124	125–250 µm	Xincheng	Various	Zhao et al. (2008)
	0.076	250–900 µm	Xincheng	Various	Zhao et al. (2008)
	1.7	<63 µm	New Bedford	Residential	USGS (2005)

1.08	63–125 μm	New Bedford	Residential	USGS (2005)
0.72	125–250 μm	New Bedford	Residential	USGS (2005)
0.26	250–2,000 μm	New Bedford	Residential	USGS (2005)
Chrysene				
ND–600	<500 μm	Bordeaux	Bridges and highway	Durand et al. (2003)
0.613	<63 μm	Xincheng	Various	Zhao et al. (2009b)
0.613	<63 μm	Xincheng	Various	Zhao et al. (2008)
0.467	63–125 μm	Xincheng	Various	Zhao et al. (2008)
0.366	125–250 μm	Xincheng	Various	Zhao et al. (2008)
0.195	250–900 μm	Xincheng	Various	Zhao et al. (2008)
1.91	<63 μm	New Bedford	Residential	USGS (2005)
1.25	63–125 μm	New Bedford	Residential	USGS (2005)
0.77	125–250 μm	New Bedford	Residential	USGS (2005)
0.36	250–2,000 μm	New Bedford	Residential	USGS (2005)
Dibenz(*a*,*h*)anthracene				
0.436	<63 μm	Xincheng, Zhejiang, China	Various	Zhao et al. (2009b)
0.436	<63 μm	Xincheng, Zhejiang, China	Various	Zhao et al. (2008)
0.208	63–125 μm	Xincheng, Zhejiang, China	Various	Zhao et al. (2008)
0.176	125–250 μm	Xincheng, Zhejiang, China	Various	Zhao et al. (2008)
0.18	250–900 μm	Xincheng, Zhejiang, China	Various	Zhao et al. (2008)
0.33	<63 μm	New Bedford, MA, USA	Residential	USGS (2005)
0.13	63–125 μm	New Bedford, MA, USA	Residential	USGS (2005)
0.12	125–250 μm	New Bedford, MA, USA	Residential	USGS (2005)
0.054	250–2,000 μm	New Bedford, MA, USA	Residential	USGS (2005)
Fluoranthene				
ND–1,400	<500 μm	Bordeaux, France	Bridges and highway	Durand et al. (2003)
2.3	<64 μm	Toronto, Ontario, Canada	Industrial	NWRI (2007)
0.8	64–2,000 μm	Toronto, Ontario, Canada	Industrial	NWRI (2007)
0.0054–0.0334	raw sweepings (<4.75 mm)	Florida, United States	Various municipal	Jang et al. (2009)
0.925	<63 μm	Xincheng, Zhejiang, China	Various	Zhao et al. (2009b)
0.925	<63 μm	Xincheng, Zhejiang, China	Various	Zhao et al. (2008)
0.698	63–125 μm	Xincheng, Zhejiang, China	Various	Zhao et al. (2008)

(continued)

Table 4 (continued)

Analyte	Concentration (ppm)	Particle fraction	Geographic location	Street type	Citation
	0.54	125–250 μm	Xincheng, Zhejiang, China	Various	Zhao et al. (2008)
	0.353	250–900 μm	Xincheng, Zhejiang, China	Various	Zhao et al. (2008)
	2.96	<63 μm	New Bedford, MA, USA	Residential	USGS (2005)
	2.55	63–125 μm	New Bedford, MA, USA	Residential	USGS (2005)
	1.75	125–250 μm	New Bedford, MA, USA	Residential	USGS (2005)
	0.71	250–2,000 μm	New Bedford, MA, USA	Residential	USGS (2005)
Fluorene					
	0.158	<63 μm	Xincheng, Zhejiang, China	Various	Zhao et al. (2009b)
	0.158	<63 μm	Xincheng, Zhejiang, China	Various	Zhao et al. (2008)
	0.139	63–125 μm	Xincheng, Zhejiang, China	Various	Zhao et al. (2008)
	0.123	125–250 μm	Xincheng, Zhejiang, China	Various	Zhao et al. (2008)
	0.073	250–900 μm	Xincheng, Zhejiang, China	Various	Zhao et al. (2008)
Indeno(1,2,3-cd)pyrene					
	0.229	<63 μm	Xincheng, Zhejiang, China	Various	Zhao et al. (2009b)
	0.229	<63 μm	Xincheng, Zhejiang, China	Various	Zhao et al. (2008)
	0.16	63–125 μm	Xincheng, Zhejiang, China	Various	Zhao et al. (2008)
	0.142	125–250 μm	Xincheng, Zhejiang, China	Various	Zhao et al. (2008)
	0.065	250–900 μm	Xincheng, Zhejiang, China	Various	Zhao et al. (2008)
	1.33	<63 μm	New Bedford, MA, USA	Residential	USGS (2005)
	0.44	63–125 μm	New Bedford, MA, USA	Residential	USGS (2005)
	0.48	125–250 μm	New Bedford, MA, USA	Residential	USGS (2005)
	0.18	250–2,000 μm	New Bedford, MA, USA	Residential	USGS (2005)
Naphthalene					
	ND–480	<500 μm	Bordeaux, France	Bridges and highway	Durand et al. (2003)
	0.322	<63 μm	Xincheng, Zhejiang, China	Various	Zhao et al. (2009b)
	0.322	<63 μm	Xincheng, Zhejiang, China	Various	Zhao et al. (2008)
	0.365	63–125 μm	Xincheng, Zhejiang, China	Various	Zhao et al. (2008)
	0.389	125–250 μm	Xincheng, Zhejiang, China	Various	Zhao et al. (2008)
	0.223	250–900 μm	Xincheng, Zhejiang, China	Various	Zhao et al. (2008)
		<63 μm	New Bedford, MA, USA	Residential	USGS (2005)
	0.03	63–125 μm	New Bedford, MA, USA	Residential	USGS (2005)

	0.015	125–250 µm	New Bedford, MA, USA	Residential	USGS (2005)
	0.013	250–2,000 µm	New Bedford, MA, USA	Residential	USGS (2005)
Phenanthrene					
	ND–990	<500 µm	Bordeaux, France	Bridges and highway	Durand et al. (2003)
	1	<64 µm	Toronto, Ontario, Canada	Industrial	NWRI (2007)
	0.5	64–2,000 µm	Toronto, Ontario, Canada	Industrial	NWRI (2007)
	1.022	<63 µm	Xincheng, Zhejiang, China	Various	Zhao et al. (2008)
	1.022	<63 µm	Xincheng, Zhejiang, China	Various	Zhao et al. (2008)
	1.035	63–125 µm	Xincheng, Zhejiang, China	Various	Zhao et al. (2008)
	0.858	125–250 µm	Xincheng, Zhejiang, China	Various	Zhao et al. (2008)
	0.587	250–900 µm	Xincheng, Zhejiang, China	Various	Zhao et al. (2008)
	1,200	<63 µm	New Bedford, MA, USA	Residential	USGS (2005)
	785	63–125 µm	New Bedford, MA, USA	Residential	USGS (2005)
	412	125–250 µm	New Bedford, MA, USA	Residential	USGS (2005)
	267	250–2,000 µm	New Bedford, MA, USA	Residential	USGS (2005)
Pyrene					
	ND–2,600	<500 µm	Bordeaux, France	Bridges and highway	Durand et al. (2003)
	1.9	<64 µm	Toronto, Ontario, Canada	Industrial	NWRI (2007)
	0.7	64–2,000 µm	Toronto, Ontario, Canada	Industrial	NWRI (2007)
	0.645	<63 µm	Xincheng, Zhejiang, China	Various	Zhao et al. (2008)
	0.645	<63 µm	Xincheng, Zhejiang, China	Various	Zhao et al. (2008)
	0.483	63–125 µm	Xincheng, Zhejiang, China	Various	Zhao et al. (2008)
	0.395	125–250 µm	Xincheng, Zhejiang, China	Various	Zhao et al. (2008)
	0.271	250–900 µm	Xincheng, Zhejiang, China	Various	Zhao et al. (2008)
	2,300	<63 µm	New Bedford, MA, USA	Residential	USGS (2005)
	1,950	63–125 µm	New Bedford, MA, USA	Residential	USGS (2005)
	1,150	125–250 µm	New Bedford, MA, USA	Residential	USGS (2005)
	550	250–2,000 µm	New Bedford, MA, USA	Residential	USGS (2005)
Bis(2-ethylhexyl)phthalate					
	0.0054–0.0149	Raw sweepings (<4.75 mm)	Florida, United States	Various municipal	Jang et al. (2009)
Di-n-butyl phthalate					
	0.0055–0.0157	Raw sweepings (<4.75 mm)	Florida, United States	Various municipal	Jang et al. (2009)

(continued)

Table 4 (continued)

Analyte	Concentration (ppm)	Particle fraction	Geographic location	Street type	Citation
Di-n-octyl phthalate					
	0.0054–0.0149	Raw sweepings (<4.75 mm)	Florida, United States	Various municipal	Jang et al. (2009)
DDD					
	0.0287–0.111	Raw sweepings (<4.75 mm)	Florida, United States	Various municipal	Jang et al. (2009)
DDE					
	0.0289–0.0494	Raw sweepings (<4.75 mm)	Florida, United States	Various municipal	Jang et al. (2009)
DDT					
	0.0251–0.461	Raw sweepings (<4.75 mm)	Florida, United States	Various municipal	Jang et al. (2009)
Alpha-BHC					
	0.05	Raw sweepings (<4.75 mm)	Florida, United States	Various municipal	Jang et al. (2009)
Beta-BHC					
	0.0281–0.0326	Raw sweepings (<4.75 mm)	Florida, United States	Various municipal	Jang et al. (2009)
Alpha-chlordane					
	0.0426–0.127	Raw sweepings (<4.75 mm)	Florida, United States	Various municipal	Jang et al. (2009)
Gamma-chlordane					
	0.0264–0.0489	Raw sweepings (<4.75 mm)	Florida, United States	Various municipal	Jang et al. (2009)
Dieldrin					
	0.0338–0.235	Raw sweepings (<4.75 mm)	Florida, United States	Various municipal	Jang et al. (2009)
Endosulfan II					
	0.039–2.410	Raw sweepings (<4.75 mm)	Florida, United States	Various municipal	Jang et al. (2009)
Endrin					
	0.0732–0.0787	Raw sweepings (<4.75 mm)	Florida, United States	Various municipal	Jang et al. (2009)

was also detected in 18 % of samples at concentrations ranging from 39 to 2,410 mg/kg (Jang et al. 2009).

4.4 Nutrients

Nitrogen and phosphorus are also contaminants of particular importance in street dust (Table 5). Urban nonpoint sources of nutrients include fertilizers in runoff from lawns, pet wastes, failing septic systems, maintenance/traction, and atmospheric deposition from industry and automobile emissions (USEPA 2005). Fertilizers may be released either slowly or rapidly, depending on how they are formulated. Rapid

Table 5 A summary of nutrient levels detected in street dust samples

Nutrients	Concentration (ppm)	Geographic location	Sample	Citation
Total Kjeldahl Nitrogen	2,400	Toronto, ON, Canada	North Bound Unswept Street 2004	NWRI (2007)
	1,055	Toronto, ON, Canada	South Bound Unswept Street 2004	NWRI (2007)
	1,133	Toronto, ON, Canada	North Bound Unswept Street 2005	NWRI (2007)
	937	Toronto, ON, Canada	South Bound Unswept Street 2005	NWRI (2007)
Total Nitrogen	1,999	Florida, USA	Highway–Commercial	Berretta et al. (2011)
	3587.7	Florida, USA	Highway–Residential	Berretta et al. (2011)
	2342.4	Florida, USA	Highway–Highway	Berretta et al. (2011)
Total Phosphorus	346–1,375	Bordeaux, France	Bridges and highway	Durand et al. (2003)
	1,333	Toronto, ON, Canada	North Bound Unswept Street 2004	NWRI (2007)
	1,185	Toronto, ON, Canada	South Bound Unswept Street 2004	NWRI (2007)
	1,313	Toronto, ON, Canada	North Bound Unswept Street 2005	NWRI (2007)
	1,335	Toronto, ON, Canada	South Bound Unswept Street 2005	NWRI (2007)
	474.6	Florida, USA	Highway–Commercial	Berretta et al. (2011)
	702.8	Florida, USA	Highway–Residential	Berretta et al. (2011)
	759.4	Florida, USA	Highway—Highway	Berretta et al. (2011)

Total Kjeldahl Nitrogen is the sum of organic nitrogen, ammoia (NH_3, and ammonium (NH_4^+)
Total Nitrogen is the sum of nitrate−N and nitrite−N and TKN

release fertilizers are of greater concern, because they are readily soluble and are easily transported into storm sewers (Brinkmann and Tobin 2001). The National Research Institute in Canada conducted a street cleaning study and found that nutrients were preferentially associated with finer street dust particles by nearly twofold on average; this observation indicates that such particles would much more likely be transported by even small amounts of stormwater runoff (NWRI 2007).

During a rainfall event, nutrients from these sources can make their way to streets in surface runoff, which in turn flows to storm sewers, and then enters aquatic receiving system where it may impair surface water quality (USEPA 2005c). Moderately high concentrations of nutrients, for example, may produce eutrophication of sensitive receiving waters, which include lakes where phosphorus is a limiting nutrient, or coastal or estuarine areas where nitrogen is limiting. A study conducted by the USEPA noted that lawns are a large source of dissolved phosphorus in some regions (USGS 2003). Fish kills can result from hypoxia and anoxia due to extreme eutrophication (USEPA 2005c). In the Gulf of Mexico, areas of chronic hypoxia have apparently resulted from increased nitrogen loads from the Mississippi River system, beginning in the 1950s (Rabalais et al. 2007). Monitoring data suggest that urban sources of nitrate are not high enough to pose a direct risk to humans, but excessive nutrient levels in receiving waters can exceed the drinking water criterion (10 mg/L for nitrate-nitrogen) (USEPA 2005c).

In a study to determine the magnitude of nutrient runoff from near shore residential lawns surrounding Lauderdale Lakes in Wisconsin, fertilizer use did not affect nitrogen concentrations in runoff (USGS 2002). However, total phosphorus concentrations in lawn runoff was directly related to phosphorus concentration of lawn soils, and test sites that used fertilizer regularly had dissolved phosphorus concentrations that were twice that from test sites that used non-phosphorus fertilizer or that did not use fertilizer (USGS 2002). This result is consistent with previous studies conducted by USGS (USGS 2003).

A study in Melbourne, Australia showed that as much as 50 % of the nutrients in street solids are associated with street dust particles smaller than 300 μm, suggesting that treatment facilities, (e.g., ponds, wetlands, and sediment basins) would remove the finer particles (down to 50 μm for total phosphorus and down to 10 μm for total nitrogen), and not just the total sediment or suspended solid load (Vaze and Chiew 2002).

5 Relevance of Street Cleaning Technologies to Ecological and Human Health Risk

5.1 Ecological Risk

More than 150 years of technological development has been invested in street cleaning as a Best Management Practices (BMP) for improving water and air quality. Few quantitative studies relative to street cleaning exist that have compared relative risks to human health or ecological risk. These gaps were highlighted in a review of 44 reports on the biological effects of highway runoff on local ecosystems (Buckler and Granato 1999). The authors cited a lack of consistency of methods and sufficient documentation for studies performed on this topic. Most studies that modeled contaminant concentrations in highway runoff predicted low acute toxicity. However, other investigations have indicated that highway runoff can impart contaminant loads to soils and sediments that affect ecosystems near discharge points (Lau et al. 2009; Lee et al. 2011; Meland et al. 2010). For example, analysis of aquatic biota revealed bioaccumulation of metals from highway runoff (Buckler and Granato 1999). One study, in which a regenerative air sweeper, a mechanical sweeper and a high efficiency regenerative air sweeper were compared, examined whether artificially generated runoff (simulated rain on a street) resulted in differential aquatic toxicity to rainbow trout and *Daphnia magna*. After exposure to simulated runoff, *D. magna* suffered less than a 50 % mortality (i.e., LC_{50}). Sweeping with the high efficiency regenerative air sweeper prior to simulating runoff resulted in reduced toxicity, but similar reductions were not observed after using the mechanical and older regenerative air sweepers (NWRI 2007).

Zhao et al. (2009a, b) conducted a risk assessment of PAHs in street dust that contributed to water quality degradation in China through surface runoff, and reported several measurements above the effects range low (ERL) and effects range median (ERM) values. Such levels respectively pose a moderate and severe impact on biota. The highest concentrations of PAHs were observed in the smallest size classes (<63 μm and 63–125 μm), and thus it is not surprising that total PAH concentrations rise as much as 43–62 % during significant rainfall events from mobilization of these smaller particles (Long et al. 1995; Zhao et al. 2009a, b).

In a study of stormwater runoff from a parking lot that received coal tar sealant nine months earlier, total PAH concentrations exceeded the National Oceanic Atmospheric Administration's (NOAA) ERM of 44.7 mg/kg, by almost twofold, compared to <5 mg/kg near non-sealed surfaces. Concentrations remained elevated three years after the initial application. PAH concentrations in dust samples on coal-tar sealed surfaces were as high as 1,192 mg/kg, compared to <2 mg/kg on non sealed surfaces (Watts et al. 2010a, b).

A risk assessment of runoff-related input of five heavy metals in several tributaries of the Yangtze River delta was also conducted and was published in a separate paper (Zhao et al. 2009a). Each of the metals (viz., chromium, copper, nickel, lead, zinc) were detected at concentrations above "severe effect screening levels" (SEL) within multiple size range categories in street dust and suspended solids in runoff during two rain events (NYSDEC 1999; Zhao et al. 2009a).

5.2 *Human Health Risk*

Re-entrainment of street dust is a major source of urban $PM_{2.5}$ and PM_{10}, which have significant impacts on human health (Amato et al. 2010a, b). An association between ambient levels of PM and mortality has been observed in numerous studies (Brunekreef and Forsberg 2005; Ruckerl et al. 2011). Recently, street sweeping has been explored as a potential method for reducing ambient PM levels, with researchers reporting mixed evidence on effectiveness (Amato et al. 2010b; Gertler et al. 2006; Keuken et al. 2010). A study conducted in Nevada showed increased aerial re-entrainment of street dust when “brush and water wash street sweepers” were used (Gertler et al. 2006). Another study conducted in the Netherlands using similar technology indicated that sweeping did not reduce non-exhaust PM emissions (Keuken et al. 2010). However, a trial conducted using a vacuum-assisted mechanical sweeper did show a small but significant reduction in ambient PM (Amato et al. 2010b). Using water for PM suppression in each of these trials may reduce one risk (human health through improved air quality) and potentially increase another (environmental through decreased water quality). The comprehensive comparison of the available technologies on reduction of PM was conducted under the City of Toronto’s Clean Roads to Clean Air initiative. The results of several studies indicated that regenerative air sweepers were most effective at reducing re-entrainment of PM (Morgan and Stevanovic-Briatico 2007).

In addition to the potential effects imposed by PM, street dust presents other hazards to human health. For example, in East China, PAHs from stormwater runoff have contaminated a potential drinking water supply (Chen et al. 2007). One hazard analysis indicated that oral ingestion of more than 100 mg/day of street dust that retained PAH concentrations could pose an unacceptable risk to human health, although ingestion of this much street dust seems unlikely (Lorenzi et al. 2011).

6 Research Needs and Conclusions

There are several variables that affect street cleaning efficiency that are essential if valid comparisons are to be made among studies. In this regard, the currently available database makes robust comparisons difficult, because the reporting of variables or test conditions in street-cleaning technology studies has been inadequate. In addition to a dearth of studies and data on the entire subject, shortfalls in addressing the following variables also exists: regional climate and soil types, street cleaning frequency, road surface type and conditions, nearby land use, and variations in technology types amongst street sweeper manufacturers. Several authors have commented on the lack of available comparative data on the effectiveness of street cleaning (Amato et al. 2010a; Kang et al. 2009; USGS 2005, 2007; Sutherland 2011). A primary objective of this paper was to provide a novel, and critical review of the efficacy of various street cleaning technologies and practices for managing environmental risks associated with stormwater and air quality. Forty-nine articles that addressed street cleaning have been examined (Table 1). Only nine recent studies reviewed herein incorporated a comparison of different types of technologies. Of these nine papers, six are empirical studies, and three are reviews that were based on computer modeling outputs. In addition, none of these studies were peer-reviewed for journal publication, and seven are government reports.

Our overall conclusions are:

1. Street dust contains significant concentrations of organic and inorganic contaminants that have the potential to negatively impact human and ecological health.

   We also reviewed the literature to quantify various constituents on street surfaces including metals, organic contaminants, and nutrients. Street cleaning in relation to environmental risk or human health has been evaluated in only a few studies (NWRI 2007; Zhao et al. 2008, 2009a, b). In several studies, concentrations of PAHs and metals in street dust, suspended solids in runoff, and bulk sediment were observed to exceed toxicity values, indicating a potential for risk to human health and the environment (Zhao et al. 2008, 2009a, b). In contrast, one set of direct tests of street dust on *Daphnia* and in an *in vitro* assay system indicated low or no toxicity (NWRI 2007). Clearly, this area deserved additional attention given the goals for stormwater management. In the context of human health, several studies noted reductions of ambient particulate matter following street cleaning, although these reductions may have been short-lived (Amato et al. 2009, 2010a, b; Chou et al. 2007; DeLuca et al. 2012; Gertler et al. 2006). In two cases, potential risks to human health were linked to soil cleanup levels (Jang et al. 2009) or estimates of high ingestion (Lorenzi et al. 2011). However, none of the available studies characterized margins of safety and/or the relative risk to human health and the environment among the available technologies and strategies for street cleaning.
2. Several types of street sweeper are available to manage street dust, including mechanical, vacuum, and regenerative air. Based on the available literature, regenerative air sweepers appear to be most effective at collecting the smallest particles and preventing re-entrainment of particulate matter.

   In this review, we examined the available literature and compared the efficacy of various street cleaning technologies. The available literature suggests that vacuum and regenerative air sweepers are more efficient at picking up smaller particles and may be better at controlling ambient PM than mechanical sweepers (NWRI 2007; USGS 2005, 2007). Generally, studies, in which sweeper technologies were compared appeared to point to regenerative air as being the superior sweeper technology; however, the variability within tests renders this initial assessment inconclusive (Sutherland 2011; TYMCO 2012b; Weston Solutions, Inc. 2010).
3. Too few data are available to make robust conclusions about what constitutes the optimal technology or practices for street cleaning as best management practice for stormwater runoff or prevention of re-entrainment of particulate matter.

   The available studies in which street sweeping technologies were compared did not follow uniform protocols, and at times were ambiguous about the nature of the equipment being tested. Customization of some of the sweepers (e.g., adjusting the proximity of the gutter brooms to the pickup head on a regenerative air unit) can affect sweeping effectiveness; unfortunately, the details of these modifications were rarely specified nor their impacts quantitatively investigated. Furthermore, most studies focused on either re-entrainment of PM or efficiency of collection from the surface, but seldom both. At present, the most comprehensive study appears to have been conducted by the City of Toronto, under their 2003 initiative "Clean Roads to Clean Air" (ETV 2011, 2012a, b, c). However, even this comprehensive testing program had its shortcomings; it was conducted in a closed environment using simulated street dust. The inherent problem with studies using a simulated test material in a controlled environment is that they usually cannot emulate real world conditions (viz., anthropogenic trash and organic debris, potholes and cracks in streets, uneven distribution across street surfaces, wet vs. dry weather conditions, flat vs. crowned streets, traffic conditions, and impervious vs. pervious pavement). Conversely, "real-world" studies must contend with the problem of numerous variables (e.g., amount of material available to pick up, wind, geographic location, weather, climate, and traffic), which serve as confounders and render defensible conclusions impossible.

Operator error, testing different sweepers at different speeds, times of the year, or places may also affect street cleaning test results (NWRI 2007). Some studies have noted improvements in stormwater quality from changes in street sweeper technology and management practices, but did not evaluate changes in street sweeper technology and management practices separately (DeLuca et al. 2012; Talend 2012). Other researchers have attempted to combine results from disparate studies in an effort to compare technology efficacy, but the many variables listed above give ample opportunities for confounding results (Sutherland and Jelen 1997; Zarriello et al. 2002). At present, the authors believe that the City of Toronto evaluation of street sweeping technologies should serve as a reasonable model for future research.

4. No standardized protocol for assessing the effectiveness of street cleaning presently exists.

It is not feasible for a single study to assess the importance of every parameter that may impact the efficiency of street dust management. The wide variation in experimental test protocols used to date prevented any rigorous assessment of comparative efficiency of the available street sweepers. Thus, we recommend establishing a standard methodology for assessing the comparative efficiency and efficacy of street cleaning technologies based on proven test protocols, establishing national standards for air and water quality though street cleaning mitigation, and establishing margins of safety for stormwater hazards and human health under multiple street cleaning strategies and technologies.

We recommend the following experimental parameters and goals for establishing a framework for evaluating street cleaning technology (Fig. 4):

1. Study environment
    (a) Ambient air quality in an enclosed area similar to the study conducted by the City of Toronto.

   (b) Various street surface conditions
      1. Smooth and rough pavement
      2. Damaged and undamaged pavement
      3. Curbed and non-curbed pavement
      4. Crowned and non-crowned surface
      5. Porous and non-porous pavement
   (c) Evaluate street cleaning technologies under various weather conditions
      1. Seasonality, particularly in drought susceptible regions and those experiencing extended winters
      2. Before and after precipitation events
      3. Cold, warm, and hot climates
2. Street dust material for testing
   (a) Simulant material such as that used in the City of Toronto study
   (b) Pure sand, silt, clay, and gravel at various moisture levels and comprising a range of particle size distributions
   (c) Collected street sweeping materials from various regions, including dirt, leaves, sticks, garbage, grass clippings, anthropogenic trash, and bulky material.
3. Street sweeping technologies, customization, and operational parameters
   (a) The effectiveness of water for dust suppression vs. flushing: efficacy and required output
   (b) Indirect environmental impacts: fuel usage and exhaust emissions per ton of material removed
   (c) Operational parameters: speed and frequency of cleaning, single vs. multiple passes, maintenance requirements, fan and broom speed and adjustment
   (d) Tandem operations

7 Summary

Street dust represents a source of dual potential risk to stormwater and air quality. It has been well documented that street dust washes into local watersheds and can degrade water quality. Research has also demonstrated that ambient particulate matter (PM_{10}), which is associated with adverse health outcomes, can arise from resuspension of accumulated street dust. Furthermore, many contaminants, including metals, are present at higher concentrations in the smallest available particles, which are more likely to be resuspended in air and stormwater runoff. Although street cleaning is listed as a best management practice for stormwater quality by the EPA, data are limited on the critical parameters (technology, environment, usage), which determine the effectiveness of any street cleaning program, particularly in the peer-reviewed literature. The purpose of the present study was to develop a comprehensive understanding of the efficacy of various street cleaning technologies and practices to protect both water quality and public health. Few studies have compared the effectiveness of street sweeping technologies to remove street dust. Unfortunately, the dearth of comprehensive data on exposure, contaminant concentrations, and efficacy of various sweeping technologies and strategies precludes developing quantitative estimates for potential risk to humans and the environment. Based on the few studies available, regenerative air street sweepers appear to provide the most benefit with regard to collection of small particles and prevention of re-entrainment. It is also clear from the available data that local conditions, climate, and specific

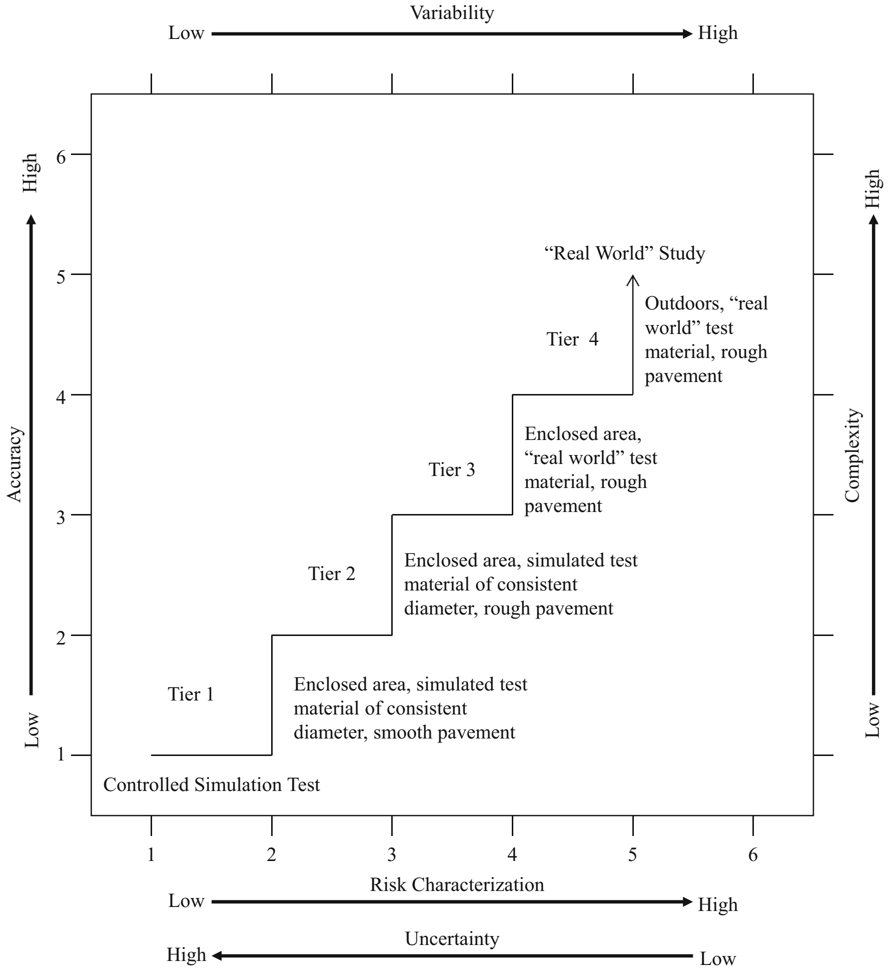


Fig. 4 A tiered risk assessment approach for evaluating efficacy of street sweeping

needs are critical determinants of the ideal street sweeping strategy (technology, frequency, speed, targeted areas, etc.). Given the critical need for protection of water and air quality in rapidly expanding urban regions (e.g., megacities), further research is necessary to develop best practices for street dust management. Herein, we provide a framework for future experimental studies to support risk-based assessments of street cleaning technologies (Fig. 4).

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Assessment of Ethylene Diurea-Induced Protection in Plants Against Ozone Phytotoxicity

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Contents

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1 Introduction

Rapid economic growth, industrialization, urbanization, and improper implementation of environmental regulations have contributed to increased tropospheric O_3 levels since preindustrial times, and this increase has produced a serious air pollution problem. Apart from being a hazardous air pollutant, O_3 has also been recognized as the third major (carbon dioxide and methane) green house gas in terms of additional radiative forcing and climate change (Forster et al. 2007). Because of its oxidative capacity, high O_3 levels in the atmosphere are detrimental to living organisms, including plants. Ozone is among the most damaging air pollutants to which plants are exposed, and produces substantive plant biomass and yield (seed weight) reductions (Thompson 1992; Agrawal et al. 2005; Manning 2005; Hassan 2006; Hassan and Tewfik 2006; Singh et al. 2009a, 2014; Wahid 2006 a, b; Sarkar and Agrawal 2010a, b; Tripathi and Agrawal 2013). The economic loss for 23 horticultural and agricultural crops from O_3 exposure was estimated to be approximately \$6.7 billion for the year 2000 in Europe (Holland et al. 2006). Wang and Mauzerall (2004) anticipated economic losses of upto 9 % for four important cereal crops (viz., wheat, rice, maize and soybean) grown in China, South Korea and Japan. To minimize such crop losses many potential antioxidants (e.g., fungicides, insecticides, growth regulators and plant extracts) have been evaluated. Among these, the systemic antioxidant, ethylene diurea, –N-[2-(2-oxo-1-imidazolidinyl) ethyl]-N′ phenylurea (popularly known as EDU) was found to be the most effective.

In this review, we address how O_3 is formed, and the phytotoxic losses it has produced over the past three decades. Moreover, we address and summarize the literature relating to efforts designed to identify antioxidants that can potentially protect vegetation from O_3 damage. We give special emphasis to the most competent and most studied of these synthetic antioxidants viz., ethylene diurea (EDU). We review EDU's effectiveness at the morphological, physiological and biochemical levels and its role in preventing yield losses in plants.

2 The Chemistry of O_3 Formation, and Its Uptake and Fate in Plants

Ozone is a secondary air pollutant that is not directly emitted to the atmosphere. It is formed by the reaction between primary air pollutants (viz., nitrogen oxides, hydrocarbons such as volatile organic carbons, carbon oxides, methane or non-methane volatile organic compounds, etc.) and solar radiation (Finlayson-Pitts and Pitts 1997). Nitrogen oxides ($NOx–NO + NO_2$) and volatile organic carbon

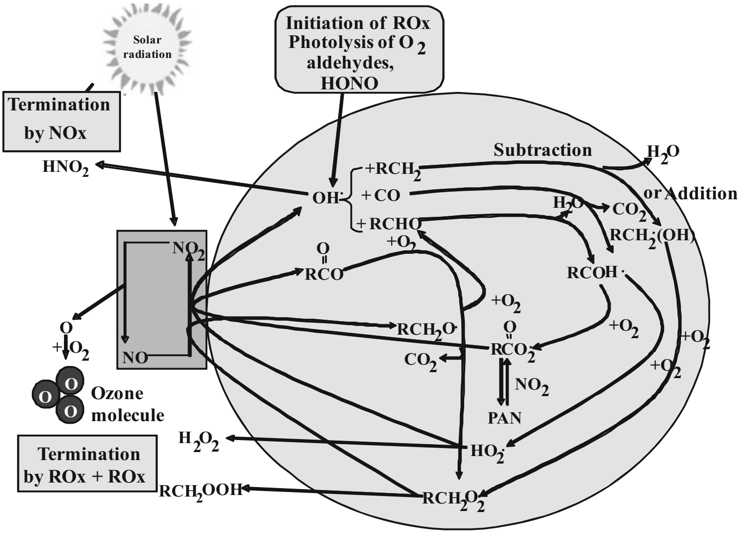

Fig. 1 The steps by which ozone is formed via photochemical processes in the troposphere (modified after Staehelin and Poberaj 2008)

(VOCs) emissions result from both natural and anthropogenic sources. Natural sources of NOx are from lightening discharges and from direct emissions from soil, whereas VOCs are released primarily from vegetation. Anthropogenic-sourced emissions of NOx result from combustion processes in motor vehicles, thermal power generation and various industrial activities. Volatile organic compounds are emitted when combustion processes are incomplete, e.g., from motor vehicle engines and from manufacturing and processing in the petrochemical industry, motor fuel production, and distribution and solvent use. In Fig. 1, we depict the steps by which O_3 is formed.

The emission of nitric oxide (NO_2) results from the reaction between nitrogen and oxygen present in atmosphere under high temperatures generally attained in the combustion chambers of engines. NO_2 thus produced is easily dissociated by the ultraviolet element of solar radiation into nitrogen monoxide and singlet oxygen ($^1O_2$). $^1O_2$ spontaneously reacts with molecular oxygen to give rise to O_3. Under these conditions, the life time of O_3 is very short, because the O_3 produced reacts with NO to produce NO_2 and O_2 again. Therefore, an equilibrium is established between O_3 formation and degradation (Lorenzini and Saitanis 2003). Alternatively, when non-methanic hydrocarbons react with NO, toxic PAN (peroxyacetyl nitrate—CH_3C (O) $OONO_2$) and other organic substances are formed. Thus, the ozone level is controlled by a complex set of photochemical reactions. There are also other chemical reactions involved in tropospheric O_3 formation that comprise a series of complex cycles, in which carbon-monoxide and VOCs are oxidized to form water vapor and carbon dioxide. The carbon monoxide oxidation results from the presence of the hydroxyl radical ($OH^{\cdot}$). The resultant hydrogen atom rapidly reacts with oxygen to give a per-oxy radical ($HO_2^{\cdot}$). Peroxy radicals then react with NO to give NO_2, which is photolyzed to give the atomic oxygen. The atomic oxygen reacts with a molecule of oxygen to form O_3. The entire process represents a chain reaction, in which O_3 becomes photo dissociated by near ultraviolet radiation to form an excited oxygen atom. Excited oxygen again reacts with water vapor to regenerate $^{\cdot}OH$ radicals, which drives the chain process. O_3 destruction also results from photochemical reactions involving NO, HO_2 or $^{\cdot}OH$. Staehelin and Poberaj (2008) reported that the NOx species restrict ozone formation by having their concentration gradually decreased from the formation of HNO_3. Ozone concentrations are influenced by precursor availability, meteorological conditions and chemical reactions over local, regional and hemispherical distances (Lenka and Lenka 2012). In low latitude regions, O_3 generally exhibits a diurnal bell-shaped pattern, reaching peak concentration during mid day and early afternoon hours and gradually decreasing during late afternoon and evening hours (Lorenzini and Saitanis 2003). Although O_3 frequently originates in urban areas, it can be transported long distances to agricultural areas via prevailing winds.

It is known that atmospheric wind turbulence facilitates O_3 transport to the plant surface (Bennett and Hill 1973). The cuticle of plant leaves act as an impermeable barrier (Kerstein and Lendzian 1989), which restricts O_3 uptake. When O_3 enters plants it does so largely via stomata. When plants are exposed to ozone, stomatal conductance is reduced (Mansfield and Pearson 1996; Castagna et al. 2001; Guidi et al. 2001; Pasqualini et al. 2002; Degl'Innocenti et al. 2003; Singh et al. 2009a). How O_3 influences stomatal conductance (Paoletti and Grulke 2005) is uncertain, although it has been postulated that O_3 may act by altering abscisic acid signaling, or by generating an active oxygen species (AOS) burst directly in guard cells (Kangasjärvi et al. 2005). After entering through stomata, O_3 reaches the sub-stomatal cavity and air spaces in the leaf. O_3 does not persist in the apoplast for long and rapidly degrades to form various reactive oxygen species (ROS) and/or reacts with biomolecules present in the cell wall, apoplastic fluid or plasma membrane (Laisk et al. 1989; Mishra et al. 2013b). Diara et al. (2005) reported that extracellular H_2O_2 accumulation is one of the earliest detectable responses to O_3 exposure. The appearance of leaf lesions has also been correlated with H_2O_2 accumulation (Pellinen et al. 1999; Wohlgemuth et al. 2002). H_2O_2 disrupts photosynthesis and activates NAD(P)H-dependent oxidase (Park et al. 1998; Rao and Davis 1999), which leads to ROS accumulation. Alscher and Hess (1993) suggested that the superoxide radical is formed and produces leaf injury under O_3 exposure. Among other ROS, the hydroxyl radical is the most reactive of oxygen species. It reacts rapidly with proteins, lipids and DNA and causes cell damage (Iqbal et al. 1996). ROS leads to a chain of reactions, which cause significant effects on the cellular metabolism of the plants. In Fig. 2, we illustrate the entry of O_3 into plant leaves, its mechanism of toxicity and consequent defense responses.

ROS cause membrane damage and deleterious effects on the normal functioning of cells. To protect against the damaging effects of ROS, plants have developed and utilize several non-enzymatic (ascorbic acid, carotenoids, glutathione, α-tocopherol) and enzymatic antioxidants (superoxide dismutase, various peroxidases, catalases,

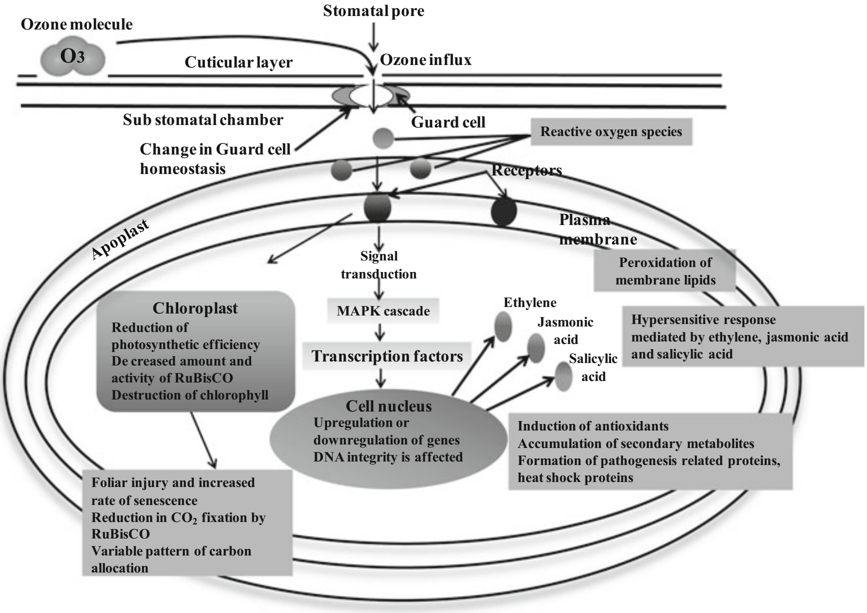

Fig. 2 Ozone uptake and its effect on cellular metabolism, signaling and cellular molecules

glutathione reductase, etc.) that exist in different cell compartments (Fig. 3). Carotenoids are non-photosynthetic pigments in leaves that help maintain the chlorophyll pool that is used against photooxidative damage by ROS. Glutathione (GSH), is the most abundant thiol in plants, and functions as an antioxidant that scavenges cytotoxic H_2O_2 and other ROS such as $OH^{\cdot}$, $O_2^{\cdot -}$ (Larson 1988). Ascorbic Acid (AA) is the most abundant low molecular weight antioxidant, is synthesized in leaf cells (Castillo and Greppin 1988; Smirnoff 2000) and forms the first line of defense against O_3 exposure (Polle et al. 1995). The ability of AA to donate electrons in a wide range of enzymatic and non-enzymatic reactions signifies it as being the main ROS detoxifying compound. In addition alpha-tocopherol is an important antioxidant for its ability to directly scavenge oxidizing radicals and to prevent chain propagation during lipid autoxidation, when plants are exposed to O_3 (Serbinova and Packer 1994).

Among enzymatic antioxidants, the primary one is superoxide dismutase (SOD), which represents a family of metalloenzymes that catalyze the dismutation of $O_2^{\cdot -}$ to H_2O_2 (Bowler et al. 1992, 1994). Another important enzyme is catalase (CAT), which exists primarily in peroxisomes and catalyzes the degradation of H_2O_2 (Willekens et al. 1995; Scandalios et al. 1997). Ascorbate peroxidase (APX), glutathione reductase (GR), monodehydroascorbate (MDHAR) and dehydroascorbate reductase (DHAR) are enzymes of the ascorbate-glutathione cycle, and help to regulate ROS formed in the presence of O_3 (Biemelt et al. 1998; Jimenez et al. 1998; Noctor and Foyer 1998).

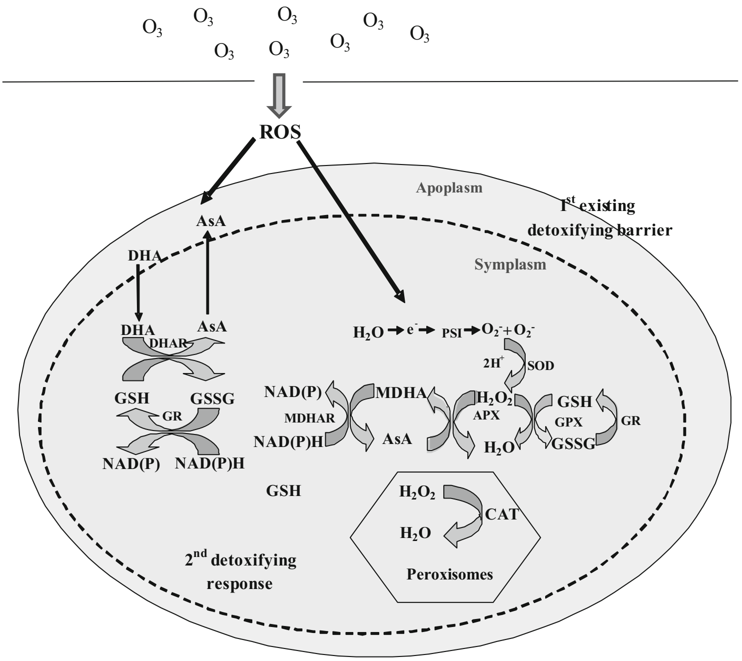

Fig. 3 The processes by which ROS are scavenged in the cells. *DHA* dehydroascorbate; *AsA* ascorbate; *DHAR* dehydroascorbate reductase; *GR* glutathione reductase; *GSSG* oxidized glutathione; *GSH* reduced glutathione; *NAD(P)* Nicotinamide adenine dinucleotide phosphate; *NAD(P)H* Nicotinamide adenine dinucleotide phosphate reduced *MDHAR* monodehydroascorbate reductase; *MDHA* monodehydroascorbate; *APX* ascorbate peroxidase; *SOD* superoxide dismutase; *GPX* glutathione peroxidase; *CAT* catalase; H_2O_2 hydrogen peroxide; *PSI* photosystem I (modified after Dizengremel et al. 2008)

Endogenous production of ROS also activates a MAP kinase cascade, which plays crucial roles in plant signal transduction pathways. MAPKs target various effector proteins, which consist of kinases, enzymes or transcription factors (Rodriguez et al. 2010). The transcription factors participate in the expression of genes that are involved in defense pathways, and in primary or secondary metabolism. Ethylene and salicylic acid are produced in plants and together foster the development of lesions and cause cell death (Castagna and Ranieri 2009). When cell death occurs, certain products of lipid peroxidation serve as substrates for synthesis of jasmonic acid. Jasmonic acid acts antagonistically and reduces ethylene-dependent ROS production and the spread of cell death (Kangasjärvi et al. 2005).

The biochemical and physiological performance of plants subjected to O_3-related oxidative stress is greatly affected and such plants display reduced growth and biomass production both of which reduce yield (Morgan et al. 2003;

Ambasth and Agrawal 2003; Biswas et al. 2008; Feng et al. 2008). Yield is of foremost concern, because reduced growth of crop plants translates directly to economic losses. Hence, yield response of plants under O_3 exposure is a major parameter that is routinely studied by plant scientists. A meta analysis of existing data has indicated that soybeans exposed to an average O_3 level of 70 ppb suffer a 24 % reduction in seed yield (Morgan et al. 2003). Moreover, Rai et al. (2007) reported that a wheat cultivar (HUW-234) grown under ambient and elevated O_3 in open top chambers suffered reduced yield. Mishra et al. (2013a) experienced the same results with two other cultivars: HUW-37 and K-9107. De Temmerman et al. (2007) found reduced root yield and altered quality of *Beta vulgaris* (sugar yield, alpha-amino-N, Na and K contents) from O_3 exposure. Intraspecific differences were observed in yield responses of rice (Ariyaphanphitak et al. 2005), wheat (Wahid 2006b; Singh and Agrawal 2009) barley (Wahid 2006a), bean (Flowers et al. 2007), potato (Piikki et al. 2004) and cotton (Zouzoulas et al. 2009) plants under different O_3 exposure regimes (Table 1). In Table 1, we summarize the results of experiments conducted to assess yield responses of different plants exposed to O_3.

3 Protectants Used to Prevent Ozone Toxicity

O_3-induced oxidative stress caused by O_3 has been identified as the major cause for plant yield losses. Hence, many chemical substances have been applied to protect plants against such losses. In recent decades, several researchers have tested various antioxidants in attempts to understand how O_3 injury may be mitigated. The types of chemicals applied have included fungicides, insecticides, growth regulators, natural plant extracts and antioxidants, among others.

Middleton et al. (1953) reported first that O_3 induced injury in pinto bean was reduced when aqueous solution of manganese (maneb) or zinc ethylenebis dithiocarbamate (zineb) was sprayed prior to O_3 fumigation. When sprayed onto poinsettia plants, the fungicides benomyl and diphenylamine (DPA) reduced O_3 injury (Manning et al. 1973a). Similar results were achieved with pinto bean (Pell 1976), potato (Carrasco-Rodriguez et al. 2005) and tobacco (Reinert and Spurr 1972) plants. Gilbert et al. (1975) reported that dust and liquid applications of DPA on apple, and dust application of DPA on bean, muskmelon and petunia also provided protection against O_3 injury. DPA was also used to quantify the effects of ambient oxidants on plants during air quality monitoring in Georgia, U.S.A. (Walker and Barlow 1974). A foliar spray of DPA at 1,000 ppm onto apple and at 1 % in bean, melon, petunia and tobacco reduced O_3 damage by 50 % or more (Lisk 1975). Carrasco-Rodriguez et al. (2005) recorded an increase in fresh biomass and tuber numbers of potato after DPA was applied.

Table 1 A summary of global studies performed to evaluate the effect of tropospheric ozone on yield of plants

Country/site	Species/cultivar	Experimental setup	Cropping condition	Parameters	O_3 conc.	Yield reductions	Reference
Belgium	*Beta vulgaris* L. Patriot	Open top chamber	Field	Root yield (t fw ha^{-1})	8 h mean, 62 ppb	14 % (2003)	De Temmerman et al. (2007)
				Sugar yield (t ha^{-1})		10.7 % (2004) 14.7 % (2003) 10.9 % (2004)	
China	*Triticum aestivum* L. and *Oryza sativa* L.	Open top chamber	Field	Grain yield $plant^{-1}$	200 ppb	80.5 and 49.1 %	Feng et al. (2003)
					100 ppb	58.6 and 26.1 %	
					50 ppb	10.5 and 8.2 %	
	Ginkgo biloba L.	Open top chamber	Field	Axial shoot and lateral shoot	80 ppb	66 and 47 %	He et al. (2006)
	Ginkgo biloba L.	Open top chamber	Field	Axial shoot and lateral shoot	80 ppb	66 and 47 %	He et al. (2006)
	Oryza sativa L. SY63 WYJ3	Open-air O_3 release system	Field	Grain yield (g per 5 hills)	Ambient (13.8–74.2 ppb, 7 h) Elevated (ambient × 1.5) Ambient (13.8–74.2 ppb, 7 h) Elevated (ambient × 1.5)	20.7 % 6.3 %	Pang et al. (2009)
	Oryza sativa L. Wujing 15 (WJ15), yangdao 6 (YD6), Shanyou 63 (SY63), Liangyoupeijiu (LYP)	O_3-FACE system	Field	Yield $plant^{-1}$ (g)	7 h mean, 56 pp for SY6, 54 ppb for YD6, 56 ppb for WJ15 and 59 ppb for LYP	17.5 % in SY6 and 15.0 % in LYP	Shi et al. (2009)
Egypt	*Gossypium hirsutum* L. Giza 65	Closed chambers	Pots	Number of open balls Seed weight (g)	70 ppb, 10 h day^{-1} for 2 weeks	23 % 15 %	Hassan and Tewfik (2006)
England	*Triticum aestivum* L. Riband	Unenclosed chamber system	Pots	Grain yield (t ha^{-1})	81 ppb, 7 h day^{-1}	13 %	Ollerenshaw and Lyons (1999)
Germany	*Solanum tuberosum* L. Hela	Closed exposure chamber	Pots	Fresh wt of tuber	24 h mean, 65 ppb 110 ppb for 4 h day^{-1}	24 % 11 %	Kollner and Krause (2000)
	Trifolium aestivum L. Nandu	Closed fumigation chamber	Pots	Thousand grain wt (g)	8 h mean for 2 weeks 65 ppb 110 ppb	12.1 % 21.2 %	Meyer et al. (2000)

Greece	*Gossypium hirsutum* L. Romanos and Allegria	Close and environment controlled chamber	Pots	Raw cotton wt. (g) Seed wt. (g)	100 ppb, 7 h day^{-1}	60.5 and 51.5 % 57.3 and 50 %	Zouzoulas et al. (2009)
India	*Triticum aestivum* L. HD 2329 *Brassica campestris* L. Pusa Jaikisan *Vigna radiata* L. Malviya jyoti	Ambient air	Pots	Yield (g $plant^{-1}$)	6 h mean Winter-10–15.4 ppb Summer-9–58.5 ppb	0.5–25.5 % 5.9–29.7 % 34.3–73.4 %	Agrawal et al. (2003)
	Vigna radiata L. Malviya jyoti	Open field	Pots	Seed wt. $plant^{-1}$ (g)	6 h mean, 9.7–58.5	22–79 %	Agrawal et al. (2006)
	Daucus carota L. Pusa kesar	Open top chamber	Field	Root wt. (g)	8 h mean, 38.4 ppb	45.3 %	Tiwari et al. (2006)
	Triticum aestivum L.	Open top chamber	Field	Yield $plant^{-1}$ (g)	8 h mean, 36.4–48 ppb	20.7 %	Rai et al. (2007)
	Oryza sativa L. Saurabh 950 and NDR 97	Open top chamber	Field	Yield (g $plant^{-1}$)	30.5–45.4 ppb	11.5 % 16 %	Rai and Agrawal (2008)
	Brassica campestris L. Kranti	Open top chamber	Field	Yield (g $plant^{-1}$)	41.6–54.2 ppb	16.4 %	Singh et al. (2009a)
	Glycine max L. PK472 Bragg	Open top chamber	Field	Yield $plant^{-1}$ (g)	70 ppb (4 h) 100 ppb (4 h) 70 ppb (4 h) 100 ppb (4 h)	20 % 33.6 % 12 % 30 %	Singh et al. (2010a)
	Triticum aestivum L. cv. HUW 510 Sonalika	Open top chambers	Field	Grain yield (g m^{-2})	12 h mean (45.3 ppb) (50.4 ppb) (55.6 ppb) (45.3 ppb) (50.4 ppb) (55.6 ppb)	20.0 % 37.0 % 46.0 % 11.0 % 25.0 % 38.5 %	Sarkar and Agrawal (2010b)
	Brassica campestris L. Sanjukta and Vardan	Open top chambers	Field	Test weight and oil content	Ambient + 10 ppb (3 h)	12.5 and 47 % 33.4 and 48.5 %	Tripathi and Agrawal (2012)
	Linum usitatissimum L. Padmini and T-397	Open top chambers	field	Weight of seeds $plant^{-1}$ and oil content	Ambient + 10 ppb (3 h)	40.5 % and 46.7 % 42.8 % and 42.5 %	Tripathi and Agrawal (2013)

(continued)

Table 1 (continued)

Country/ site	Species/cultivar	Experimental setup	Cropping condition	Parameters	O_3 conc.	Yield reductions	Reference
	Triticum aestivum L. HUW-37 and K-9107	Open top chambers	Field	Yield (g plant^{-1})	Ambient + 10 ppb (4 h)	First year 39 % and 12.4 % Second year 40.8 % and 14 %	Mishra et al. (2013a)
	Trifolium alexandrium L. Bundel, Wardan, JHB-146, Fahli, Saidi and Mescavi	Open top chambers	Field	Total biomass (g plant^{-1})	Ambient + 10 ppb (6 h)	13.5 % 18.2 % 9.1 % 4.9 % 6.5 % 4.4 %	Chaudhary and Agrawal (2013)
	Zea mays L. HQPM1 and DHM117	Open top chambers	Field	Weight of kernels plant^{-1}	Ambient Ambient + 15 ppb (5 h) Ambient + 30 ppb (5 h)	4.0 % 7.2 % 10.1 % 5.5 % 9.5 % 13.8 %	Singh et al. (2014)
Ireland	*Triticum aestivum* L. Promessa	Open top chamber	Field	Dry wt. of grains plant^{-1} (g)	Ambient + 50 ppb 4 h day,4 days week Ambient + 25 ppb 6 h day, 5 days week Ambient + 25 ppb 6 h day, 5 days week Ambient + 50 ppb 3 h day, 5 days week	53.5 % NS 3.2 % 16.8 %	Finnan et al. (1996)
Italy	*Triticum aestivum* L. Taylor's Horticultural, Lingua di Fuoco and Saluggia	Open top chamber	Field	Dry wt of pod (t ha^{-1})	7 h mean, 50 ppb	31.5 % 28.6 % 30.8 %	Schenone and Lorenzini (1992)
	Trifolium repens L. Regal NC-R (O_3 resistant) NC-S (O_3 sensitive)	Ambient field	Pots	Biomass	7 h mean, 49–70 ppb	20–60 % (in comparison to NC-S)	Fumagalli et al. (2003)
	Glycine max L.	Open top chamber	Field	Grain yield (kg m^{-2})	60–70 ppb	47 %	Bou Jaoude et al. (2008)
	Phaseolus vulgaris L. Borlotto Nano Lingua di Fuoco	Open top chamber	Field	Seed wt plant^{-1} (g)	4,675 ppb h (cumulative exposure)	40.6 %	Gerosa et al. (2009)

Malaysia	*Oryza sativa* L. MR 84 and MR 185	Open top chambers	Field	Yield plant^{-1} (g)	8 h mean, 32.5 ppb	3.4 % in MR 84 and 6.3 % in MR 185	Ishii et al. (2004)
Pakistan	*Oryza sativa* L. IRRI-6 and Basmati-385	Open top chamber	Pots	Grain yield plant^{-1} (g)	6 h mean, 35.6 ppb	37 % and 42 %	Wahid et al. (1995)
	Hordeum vulgare L. Haider-93, Haider-91, Jou-87 and Jou-85	Open top chamber	Pots	Seed wt plant^{-1} (g)	8 h mean, 71 ppb	13 %, 30 %, 34 % and 44 %	Wahid (2006a)
	Triticum aestivum L. Inqilab-91, Punjab-96 and Pasban-90	Open top chamber	Pots	Grain yield Plant^{-1} (g)	8 h mean, 72 ppb	18 %, 39 % and 43 %	Wahid (2006b)
	Vigna radiata L. M-28 and 6601	Open top chamber	Ambient Field	Seed yield plant^{-1} (mg)	41–73 ppb	47.1 and 51.1 %	Ahmed (2009)
Saudi Arabia	*Triticum aestivum* L. Giza 68 *Vicia faba* L. Lara *Phaseolus vulgaris* L. Giza 3 *Pisum sativum* L. Perfection	Open field	Pots	Seed yield pot^{-1} (g)	77–166 ppb	9–46 % 13–33 % 20–45 % 3–30 %	Ali et al. (2008)
Spain	*Solanum tuberosum* L. Desiree	Green house	Field	Commercial tuber production	12 h mean, 25.8 ppb 42.5 ppb	53 % 65 %	Calvo et al. (2009)
Sweden	*Solanum tuberosum* L. Bintje	Open top chamber	Field	Tuber Fresh wt (g)	12 h mean, 43 ppb	11.4 %	Persson et al. (2003)
	Solanum tuberosum L. Bintje and Kardal	Open top chamber	Field	Dry mass of tubers Number of tubers	31 and 57 ppb	20 and 30 % (Bintje) 27.8 and 20.4 % (Kardal) 7.7 and 19.2 % (Bintje) 11.4 and 11.4 % (Kardal)	Piikki et al. (2004)
Switzerland	*Triticum aestivum* L. Albis	Open top chamber	Field	Grain yield (t ha^{-1})	Mean 7 h day^{-1} 1989 35.7 ppb 49.5 ppb 62.2 ppb 1990 38.7 ppb 55.6 ppb 71.4 ppb	4.7 % 18.9 % 29.3 % 6.9 % 14.7 % 32.1 %	Fuhrer et al. (1992)

(continued)

Table 1 (continued)

Country/site	Species/cultivar	Experimental setup	Cropping condition	Parameters	O_3 conc.	Yield reductions	Reference
Thailand	*Oryza sativa* L. Chainat1 Suphanburi 1 Suphanburi 60 Suphanburi 90 Klongluang 1 Pathumthani 1 Gorkor 15 Khowdokmali 105 *Glycine max* L. Sorjor 4 Chaing Mai60	Closed top chamber	Field	% filled seed $year^{-1}$	24.9 ppb	15.9 % 12.3 % 5.6 % 8.2 % 5.7 % 16.6 % 8.2 % 2.7 % 16.3 % 17.7 %	Ariyaphanphitak (2004)
	Oryza sativa L. Klongluang 1, Pathumthani 1, Gorkor 15 and Khowdokmali 105	Closed chamber	Pots	Filled seed ear^{-1}	150 ppb, 7 h day^{-1}	30–78 %	Ariyaphanphitak et al. (2005)
United Kingdom	*Brassica napus* L. Eurol	Controlled environment chambers	Field	Seed yield ($t\ ha^{-1}$)	77–80 ppb for 49 days	14 %	Ollerenshaw et al. (1999)
USA	*Glycine max* L. Merr. Davis	Open top chamber	Field	Seed yield	104 ppb, 7 h day^{-1}	50 %	Unsworth et al. (1984)
	Glycine max L. Forrest and Essex	Open top chamber	Field	Seed yield ($g\ m^{-2}$)	62.4 ppb, 7 h day^{-1} 5 days a week	32 % 10 %	Chernikova et al. (2000)
	Glycine max L.	–	Field	Seed weight $plant^{-1}$ (g)	70 ppb	24 %	Morgan et al. (2003)
	Glycine max L. Merr.	Open top chamber	Field and pots	Total seed wt	75 ppb (1999) 67 ppb (2000)	23.9 % (field) 27.2 % (pot) 38.9 % (field) 41.0 % (pot)	Booker and Fiscus (2005)
	Phaseolus vulgaris L. S156, R 123 and R 331	Environmentally controlled field chambers	Pots	Seed yield ($g\ plant^{-1}$)	60 ppb	77 %, 19 % and 35 %	Flowers et al. (2007)
	Oryza sativa L.	Open top chamber	Field	Seed yield (g)	62 ppb	24 %	Ainsworth (2008)
	Oryza sativa L	Open top chamber	Pots	Seed wt ($g\ m^{-2}$)	73–77 ppb	17 % (1997) 14 % (1998)	Reid and Fiscus (2008)

NS not significant, *t* tonne, *fw* fresh weight, *ha* hectare

Foliar application of two modern fungicides (azoxystrobin and epoxiconazole) prior to fumigation with injurious doses of O_3 (150–250 ppb; 5days; 7 h/day) reduced visible injury by 50–60 % in spring barley by inducing an antioxidative defense system (Wu and Tiedemann 2002). To prevent O_3 injury benomyl is the most studied benzimidazole derivative fungicide (Manning et al. 1972, 1973a, b, c; Manning and Vardaro 1973a, b). In the 1970s, the discovery that benomyl can protect plants against O_3 injury opened new investigations because carboxin had similar beneficial effects in reducing O_3 injury in plants. However, benomyl was more effective than carboxin, because, to achieve equal protection of benomyl carboxin required a dose that was nearly phytotoxic (Rich et al. 1974; Taylor and Rich 1974; Papple and Ormrod 1977). Hofstra et al. (1978) found that benomyl and carboxin were equally effective in causing yield recovery in navy beans exposed to O_3. Recently, the fungicide 'strobi' conferred the best protective effects on O_3 sensitive clover and tobacco among the studied modern agrochemicals (Blum et al. 2011).

Insecticides have also been applied to achieve protection, although their application has been limited to areas where crop loss does not result from insects. Of ninety chemicals tested, only five showed significant protection from O_3 injury (Koiwai et al. 1974). These five were: 3, 4-methylenedioxyphthaldehyde, benzimidazole, safroxane, xanthone and piperonyl butoxide. Phytohormones such as auxins, cytokinins and abscisic acid (ABA) acted like antioxidants in reducing O_3 injury in plants (Kurchii 2000; Pauls and Thopson 1982; Verbeke et al. 2000). Abscisic acid (ABA), a chemical that induces stomatal closure reduced the O_3 injury in bean (Fletcher et al. 1972). Protective effects from the application of N-6-benzyladenine (BA), gibberellic acid (GA) and indole acetic acid (IAA) against O_3 were observed to occur in radish, and BA was the most effective protectant of the three (Adedipe and Ormrod 1972). Runeckles and Resh (1975) reported that the cytokinins in BA and kinetin stimulated leaf growth and reduced chlorophyll loss, which generally is the most susceptible constituent to O_3 attack.

Ascorbic acid and its salt are effective in minimizing the detrimental effects of O_3 in bean, celery, lettuce, barley, citrus and petunia (Freebairn 1960; Freebairn and Taylor 1960; Dass and Weaver 1968; Lee et al. 1990; Macher and Wasescha 1995). In contrast, Siegel (1962) reported that ascorbic acid failed to provide any appreciable protection to cucumber plants after O_3 exposure. Ozoban, an isomer of ascorbic acid reduced the photosynthetic rate in a low-O_3 environment, but was harmful to chloroplastic plant pigments that were exposed to elevated levels of O_3 (Kuehler and Flagler 1999). Agrawal et al. (2004) studied three wheat cultivars and showed that an ascorbic acid spray improved biochemical parameters and increased biomass and yield, but did not impart any significant change in stomatal conductance. How application of ascorbate induces plant-resistance to O_3 stress is still unclear (Didyk and Blum 2011). Among all protectant types, the performance of ethylene diurea (EDU) is the best documented, and has been suggested to be the most efficient research tool for analyzing O_3-induced stress in a variety of plants (Manning 1992).

4 Ethylenediurea (EDU) as a Protectant to Prevent Phytotoxicity

The positive effect of ethylenediurea (EDU, N-[2-(2-oxo-1-imidazolidinyl) ethyl]-N′-phenylurea) on plant productivity against ambient O_3 was first reported by Carnahan et al. (1978). It is specific in suppressing O_3 injury and has no effects on peroxylacetylnitrate (PAN) and sulphur dioxide (SO_2)-induced injury (Cathey and Heggestad 1982a; Lee et al. 1992). EDU consists of two urea moieties having an imidazole ring (urea) and a phenylurea ring; these rings are joined by an ethylene group (Fig. 4). An investigation of the relative effectiveness of EDU and constituent amounts of urea and phenylurea in EDU in prevention of O_3 injury was performed by Godzik and Manning (1998). They suggested that urea did not impart protection against O_3 injury, although phenylurea reduced O_3 injury significantly. Urea treatment was clearly not as effective as the treatment with EDU. However, when O_3 exposed plants were treated with 300 ppm EDU or phenylurea, both compounds equally prevented O_3 injury. No published reports addressed the role that the nitrogen present in EDU had in protecting plants from O_3. EDU neither acts as a nutrient, nor shows any pesticidal or plant regulatory effects (Manning 1992). Paoletti et al. (2008) investigated the biochemical leaf responses in EDU infused ash trees and reported that EDU did not contribute nitrogen as a fertilizer (*Fraxinum excelsior*). Lee and Chen (1982) reported cytokinin-like activity of EDU and indicated that EDU retarded the breakdown of chlorophyll, protein and RNA in O_3-sensitive tobacco leaf discs. Whitaker et al. (1990) studied the role of EDU in protecting foliar lipids and concluded that EDU conferred O_3 tolerance by inducing enzyme systems involved in the elimination of activated oxygen species and free radicals.

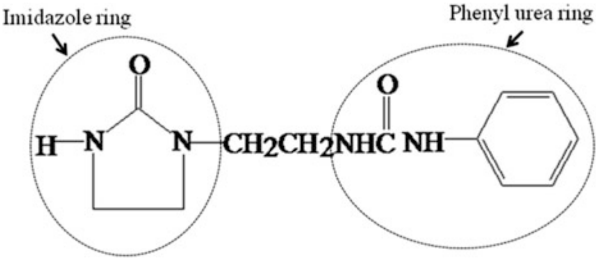

Fig. 4 Structural formula of N-[2-(2-oxo-1-imidazolidinyl) ethyl-]-N′-phenyl urea, (abbreviated as EDU, or ethylene diurea)

4.1 Methods and Timing of EDU Application

In most experiments, EDU has mainly been applied as a soil drench or foliar spray (Table 2). Other EDU application methods have included stem injection and gravitational infusion (Fig. 5), and these have commonly been used in trees and other woody species (Roberts et al. 1987; Ainsworth and Ashmore 1992; Ainsworth et al. 1996; Bortier et al. 2001; Paoletti et al. 2007). Bortier et al. (2001) found that stem injection of EDU in *Populus nigra* was effective in preventing O_3-induced visible injury, accelerated senescence, and led to significant increases in diameter and height of O_3 exposed trees. Ainsworth et al. (1996) reported similar results with two clones of hybrid poplar that showed incremental chlorophyll content increases after EDU treatment. Paoletti et al. (2007) found a significant reduction in O_3-induced foliar injury in ash trees when EDU (300 and 450 ppm) was applied as a gravitational infusion into the trunk. Percent O_3 injury was reduced to 3 % in EDU-infused trees as compared to control plants that manifested 13 % injury on the leaf surface.

Although EDU is usually applied as a soil drench to protect against O_3 injury in plants, the applied EDU may accumulate in soil and produce subsequent toxicity. Some argue therefore that foliar applications are safer than drenching. Moreover, utilizing drenching on a large scale basis, or in large fields, is not always feasible at all stages of plant development, particularly in non-row crops such as hay and broadcasted cereals. In addition, large amounts of EDU are required when used at the field scale.

Surface applications are an alternative application method, but have their own drawbacks, such as being dependent on precipitation to effect the chemical uptake by roots. Feng et al. (2010) performed a meta analysis, which indicated that EDU applied as a soil drench significantly ameliorated plant growth suffering from O_3 stress. In contrast, after EDU was applied as a foliar spray, only a few parameters showed significant positive effects of EDU. Alternative application approaches, like stem injections and gravitational infusion of EDU solution are not possible for delicate plants like vegetables and cereal crops.

Application timing of EDU is critical for achieving maximum protection to plants against O_3 injury. Weidensaul (1980) found that pinto beans were best protected from O_3 injury when EDU was applied 3–7 days prior to O_3 exposure, but afforded no protection to leaves that were not formed at the time of chemical application. McClenahen (1979) reported almost complete protection from O_3 injury in *Fraxinus americana* and *Prunus serotina* that received up to 300 ppb EDU weekly during the seedling stage. Using ^{14}C-EDU (Roberts et al. 1987) and a HPLC technique (Regner-Joosten et al. 1994), Gatta et al. (1997) established that EDU translocation was primarily acropetal, probably via the xylem stream and that EDU remained in the apoplastic region of leaves for more than 10 days, without being redistributed to newly formed leaves. Carnahan et al. (1978) documented that EDU was not translocated to newer or untreated leaves, which suggested that repeated applications were needed to assure continuous protection from O_3 injury.

Table 2 Effects of ethylene diurea (EDU) treatment on shoot and root length, number of leaves and leaf area of plants grown under ozone exposure

Country/ site	Species/ cultivar	EDU application	EDU dose/con.	O_3 con.	Application duration	Shoot length	Root length	Number of leaves	Leaf area	Biomass	Reference
Belgium	*Populus nigra* L. Wolterson	Stem injection	5 mg/ plant	32 ppb (8 h)	14 days	5.3 % (↑)	–	–	–	9 % (↑)	Bortier et al. (2001)
China	*Oryza sativa* L. *Triticum aestivum* L	Foliar spray	150 ppm 300 ppm 450 ppm 150 ppm 300 ppm 450 ppm	Often exceeding 40 ppb	7 days	– – – – – –	– – – – – –	– – – – – –	– – – – – –	NS NS NS NS NS NS	Wang et al. (2007)
Egypt Suburban Rural	*Raphanus sativus* L. *Brassica rapa* L. *Raphanus sativus* L. *Brassica rapa* L.	Soil drench	500 ppm/ 200 mL	54.8 ppb (6 h) 66.9 ppb (6 h)	10 days	– – – –	– – – –	– – – –	– – – –	25 % (↑) 4.2 % (↑) 20.5 % (↑) 13.2 % (↑)	Hassan et al. (1995)
Rural	*Trifolium alexandrinum* L. Messkawy	Soil drench	50 ppm/ 200 mL 100 ppm/ 200 mL 150 ppm/ 200 mL 200 ppm/ 200 mL	88 ppb (8 h day^{-1})	10 days	– – – –	– – – –	– – – –	– – – –	21.4 % (↑) 71.4 % (↑) 92.8 % (↑) 95.2 % (↑)	Hassan et al. (2007)
Urban Peri-urban	*Lycopersicon esculentum* L. Pusa Ruby	Soil drench	400 ppm	88.41 ppb (5 h) 89.53 ppb (5 h)	12 days	21.1 % (↑) 24.3 % (↑)	30 % (↑) 11.4 % (↑)	14.5 % (↑) 15.9 % (↑)	– – –	31.5 % (↑) 33.8 % (↑)	Varshney and Rout (1998)

Peri-urban				89.98 ppb (5 h)		24.8 % (↑)	11.2 % (↑)	16.6 % (↑)		36.6 % (↑)	
Urban	*Triticum aestivum* L HD 2329 HUW 234 HUW 468	Soil drench	500 ppm/ 250 mL	29.2 ppb (6 h)	7 days	7.5 % (↑) 13.7 % (↑) 5.4 % (↑)	15.9 % (↑) 16.7 % (↑) 33.2 % (↑)	13.6 % (↑) 21 % (↑) 16.6 % (↑)	(↑) (↑) (↑)	(↑) (↑) (↑)	Agrawal et al. (2004)
Suburban	*Vigna radiata* L. Malviya Jyoti	Soil drench	500 ppm/ 500 mL	32.6–35.2 ppb (8 h)	7 days	12.3 % (↑)	(↑)	16.8 % (↑)	30 % (↑)	24 % (↑)	Agrawal et al. (2005)
Suburban	*Triticum aestivum* L. M 234 M 533	Soil drench	150 ppm/ 200 mL 300 ppm/ 200 mL 450 ppm/ 200 mL 150 ppm/ 200 mL 300 ppm/ 200 mL 450 ppm/ 200 mL	Often exceeding 40 ppb (8 h)	10 days	– – – – – –	– – – – – –	46.5 % (↑) 63.8 % (↑) 32.7 % (↑) 20.9 % (↑) 22.4 % (↑) 8.9 % (↓)	7.9 % (↑) 8.7 % (↑) 2.6 % (↓) 22 % (↑) 26.6 % (↑) 23 % (↑)	48.1 % (↑) 44.6 % (↑) 62.7 % (↑) 25.5 % (↑) 40.4 % (↑) 60.5 % (↑)	Tiwari et al. (2005)
Suburban	*Beta vulgaris* L. Allgreen	Soil drench	300 ppm/ 200 mL	52.1–73.2 ppb (8 h)	10 days	19.7 % (↑)	–	28 % (↑)	18.3 % (↑)	31.8 % (↑)	Tiwari and Agrawal (2009)

(continued)

Table 2 (continued)

Country/ site	Species/ cultivar	EDU application	EDU dose/con.	O_3 con.	Application duration	Shoot length	Root length	Number of leaves	Leaf area	Biomass	Reference
Suburban	*Triticum aestivum* L HUW234 HUW468 HUW510 PBW343 Sonalika	Soil drench	400 ppm/ 100 mL	35.3–54.2 ppb (8 h)	12 days	12.8 % (↑) 15.2 % (↑) 17.3 % (↑) 9.2 % (↑) 2.3 % (↑)	8 % (↑) 27.2 % (↑) 12.7 % (↑) 12.5 % (↑) 6.3 % (↑)	23.7 % (↑) 41.5 % (↑) 28.5 % (↑) 29.4 % (↑) 14.3 % (↓)	3.3 % (↑) 52 % (↑) 47.9 % (↑) 40.4 % (↑) 28.8 % (↑)	54.5 % (↑) 47.6 % (↑) 31.3 % (↑) 10.9 % (↑) 2.6 % (↑)	Singh and Agrawal (2009)
Rural	*Vigna radiata* L. Malviya Janpriya	Soil drench	400 ppm/ 100 mL	52.9–64.5 ppb (12 h)	10 days	22.8 % (↑)	18.7 % (↑)	(↓)	(↑)	24.9 % (↑)	Singh et al. (2010b)
Pakistan Punjab Post-monsoon Rural roadside Rural Suburban Pre-monsoon Rural roadside Rural Suburban	*Glycine max* L. NARC-1	Soil drench	400 ppm/ 600 mL	48 ppb (6 h) 48 ppb (6 h) 40 ppb (6 h) 70 ppb (6 h) 75 ppb (6 h) 63 ppb (6 h)	10 days	20.7 % (↑) 12.4 % (↑) 8.1 % (↑) 53.9 % (↑) 47.6 % (↑) 29.8 % (↑)	– – – – – –	43.7 % (↑) 30.5 % (↑) 17.7 % (↑) 49.2 % (↑) 37.9 % (↑) 14.8 % (↑)	– – – – – –	– – – – – –	Wahid et al. (2001)

USA	*Pinus taeda* L.	Foliar spray	150 ppm 300 ppm 450 ppm	40–70 ppb	14 days	– (↑) (↑)	– – –	– – –	– – –	– (↑) (↑)	Manning et al. (2003)
	Rudbeckia laciniata L.	Foliar spray	200 ppm 400 ppm 600 ppm	32–38 ppb (12 h)	7 days	– – –	– – –	– – –	– – –	11.9 % (↓) 29.6 % (↓) 22 % (↓)	Szantoi et al. (2009)

(↑) = increase, (↓) = decrease, *NS* not significant

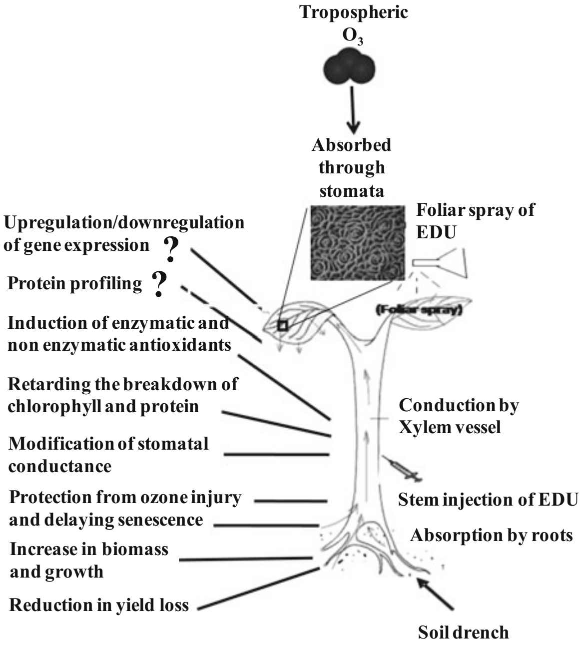

Fig. 5 Flow diagram showing influx of O_3 through stomata and the possible mechanism by which EDU protects plants against O_3 stress

EDU is a systemic antioxidant, and is not redistributed to new tissues; hence, repeated applications are indeed needed to maintain continuous levels of protection (Weidensaul 1980; Regner-Joosten et al. 1994). Depending on plant sensitivity to $O_{3,}$ Manning et al. (2011) suggested foliar spraying with EDU at weekly or bi-weekly intervals. Paoletti et al. (2009) reviewed the use of EDU on Italian crops and trees and reported that amelioration of visible O_3-induced injury was obtained by regularly applying EDU at 2 or 3 week intervals.

4.2 Application Dose of EDU

To determine the optimal EDU dose that gives the best protection against $O_{3,}$ without producing side effects, the application rates of EDU must be standardized for each species. Carnahan et al. (1978) was first in trying to standardize the EDU application rates (0–500 ppm) adequate to protect pinto beans from O_3 exposure effects. EDU at 500 ppm was seen as an application rate that optimally protected plants from acute O_3 injury (Carnahan et al. 1978; Weidensaul 1980; Cathey and Heggestad 1982a, b, c). Cathey and Heggestad (1982a, b), in an exposure/response screening trial, confirmed that a foliar spray or soil drench of 500 ppm EDU provided optimal protection for 4 cultivars of petunia and 44 species of herbaceous plants.

EDU has been used as a survey tool for measuring O_3 effects under field conditions. Postiglione and Fagnano (1995) studied ambient O_3 effects on lettuce, subterranean clover, bean and tomato by using EDU. Fumagalli et al. (1997) used EDU (150 ppm) in an ambient O_3 environment, coupled with open-top chambers to study the effects of O_3 on white clover in the Milan region of Italy. In both studies, researchers found that EDU had positive effects in reducing O_3 injury to the respective tested plants.

Manning (1988, 1992, 1995), Kostka-Rick and Manning (1993b, c), Tiwari et al. (2005) and Singh et al. (2010a) performed studies that yielded appropriate dose-responses. Cathey and Heggestad (1982c) observed that a 500 ppm rate of EDU was most appropriate for woody plant protection. Later studies utilized repeatitive (weekly or biweekly) EDU applications to protect plants against chronic O_3 exposures (Clarke et al. 1983, 1990; Hofstra et al. 1983; Bambawale 1986; Heggestad 1988; Brennan et al. 1990; Legassicke and Ormrod 1981; Toivonen et al. 1982). Additional studies utilized variable dosages of EDU (300–500 ppm) to protect plants from acute and chronic O_3 levels, viz., 300 ppm (Hassan 2006), 400 ppm (Wahid et al. 2001; Singh and Agrawal 2009; Singh et al. 2009b) and 500 ppm (Agrawal et al. 2004, 2005). Tiwari et al. (2005) and Wang et al. (2007) conducted dose-response studies on wheat. Results were that ambient O_3-exposed plants treated with 300 ppm EDU, but not at 200 and 400 ppm, displayed various improved growth characteristics. EDU treatment at 450 ppm caused significant increments in growth parameters of Loblolly pine (Manning et al. 2003). In contrast, Szantoi et al. (2009) reported a significant decrease in root and total biomass of *Rudbeckia laciniata* as EDU concentrations increased (200, 400 and 600 ppm), although the percentage of leaf injury was reduced. After performing a meta analysis study, Feng et al. (2010) suggested that EDU applied as a soil drench at a concentration range of 200–400 ppm had the most positive effects on field grown crops.

4.3 Effectiveness of EDU and Its Toxicity

Most EDU studies did not show toxic effects from EDU treatment, when applied at optimal concentrations to address O_3 stress. No protective effects of EDU were reported when it was applied under the following conditions: (i) applied to a O_3-resistant cultivar, (ii) applied on plants grown in O_3-free air; however, an EDU application manifested toxic effects on plants when the EDU concentration was very high. Legassicke and Ormrod (1981) and Foster et al. (1983) found that EDU treatment did not increase plant yield in the O_3 resistant ‘New Yorker’ tomato cultivar and ‘White Rose’ potato cultivar. Clarke et al. (1983) observed a similar result in the ‘Green Mountain’ potato cultivar. Elagoz and Manning (2002) used one sensitive (S156) and another resistant (R123) variety of bean to validate that EDU caused a side-effect on the resistant variety. A significant increase in the above ground biomass (pod and seed weight) of an EDU-treated sensitive variety was observed. In contrast, significant reductions in the above parameters were observed in R123 (a resistant variety). A similar response trend was observed in sensitive and resistant varieties of tobacco (Godzik and Manning 1998).

No significant yield increases of an O_3-sensitive potato grown in O_3-free air were recorded. In contrast, increasing application frequency resulted in over-dosing and caused side-effects from EDU treatment on root and shoot biomass (Foster et al. 1983; Bisessar and Palmer 1984). No significant differences in chlorophyll content, foliar injury, plant height, pod number and seed yield of soybean were observed in EDU-treated and untreated plants, when O_3 was absent (Greenhalgh et al. 1987). Hassan et al. (1995) also reported insignificant differences in root, shoot, total biomass and the root-shoot ratio in radish and turnip grown in filtered chambers, in the presence or absence of EDU. No significant difference between CF (controlled filtered air)/-EDU and CF (controlled filtered air)/+EDU treatments were observed for the measured parameters (healthy leaf number, injured leaf number, green stem, root and leaf biomass, leaf area index, total nitrogen content in plant parts, total dry biomass, grain yield, seed weight, seed protein, seed oil, etc.) of soybean plants (Ali and Abdel-Fattah 2006). Other literature reports suggested that EDU did not affect plant growth and productivity in the absence of $O_{3,}$ e.g., for soybean (Kostka-Rick and Manning 1993b) and tobacco (Godzik and Manning 1998).

EDU has been reported to be toxic at high concentrations. In *Nicotiana tabacum*, foliar sprays did not cause visible injury, but soil drench applications at 500, 1,000 and 2,000 ppm caused phytotoxicity in Bel-W3 seedlings (Lee and Chen 1982). Eckardt and Pell (1996) reported complete protection (from a lower EDU dose of 15 ppm) of accelerated foliar senescence that had been induced by O_3-exposure in potato. Higher dosages of EDU (45 and 75 ppm) were associated with leaf curling and marginal necrosis. Szantoi et al. (2009) found a linear reduction in root and total biomass of *R. laciniata* as EDU levels increased. Weidensaul (1980) suggested that higher concentrations of EDU (800–5,000 ppm) applied as foliar sprays are more effective in preventing foliar injury in pinto bean, an O_3 sensitive plant. However, when applied in excess, negative effects on plant physiology were reported by Bennett et al. (1978) and Heagle (1989). Heggestad (1988) reported that weekly applications of a 500 ppm EDU spray on four cultivars of field-grown sweet corn produced phytotoxicity, reduced growth and yield. It is therefore evident that the efficacy of EDU is more apparent in O_3 sensitive cultivars when applied at optimum doses, but only when O_3 stress exists. Resistant varieties gain no benefit from EDU applications. However, low concentrations of EDU that do not affect plant growth can nonetheless provide protection from visible O_3 injury.

5 EDU and Its Modes of Action

In our review of the literature, we sought to better understand the biochemical, growth and metabolic roles of EDU's action and mechanism. In this section we have compiled the results that bear on these points below.

5.1 Effects of EDU on Growth Characteristics and Biomass Accumulation

Plant growth is a complex phenomenon that results from integration and coordination of various physiological and biochemical processes that are genetically controlled and greatly influenced by various environmental factors. Ozone is a strong oxidant that causes many effects in plant species, and in particular cultivars of those species, often at the developmental stage. EDU provides O_3 tolerance by modifying several plant processes, and ultimately protects plants from O_3 damage. Kostka-Rick and Manning (1992) reported that EDU treatment increased root biomass of *Raphanus sativus* (radish) during early growth stages, but imparted no protective effects at later stages. However, EDU treatment did not alter shoot or total biomass of radish. Kostka-Rick and Manning (1993a) reported no growth-related changes in EDU-treated plants of *Phaseolus vulgaris*, whether grown in synthetic substrates or in the field, although symptoms related to EDU toxicity were observed at a high concentration. A general conclusion of all researchers is that EDU treatment reduced or delayed O_3 injury symptoms in plant foliage, and also delayed the senescence process.

In a dose–response study conducted in closed chambers on O_3-exposed *P. vulgaris*, increasing EDU treatment levels (150–300 mg L^{-1}) significantly helped plants to accumulate more stem, leaf and total biomass, in comparison to plants not treated with EDU (Astorino et al. 1995). Brunschon-Harti et al. (1995a) reported reduced growth suppression by O_3 in the form of higher leaf, root and shoot dry weight of EDU-treated plants of *P. vulgaris*. However, Ainsworth et al. (1996) did not find a significant difference in growth characteristics of two clones of hybrid poplar, after EDU was injected in stems at rates of 250 ppm and 1,000 ppm. However, a significant reduction in O_3 injury, decreasing senescence of leaves and an induction in chlorophyll content were observed.

In Table 2, we have summarized studies conducted to assess the impact of O_3 on growth characteristics and biomass accumulation in plants that were subjected to EDU applications. Agrawal et al. (2005) noticed significant increases (viz., 12.3, 16.8, 30 and 24 %, respectively in shoot length, number of leaves, leaf area and total biomass) of EDU-treated (500 ppm) mungbean plants, compared to non-EDU treated plants (Table 2). Singh et al. (2010b) reported that EDU given as soil drench (400 ppm) to mungbean caused significant growth parameter increases, including biomass, but noted a reduction in number of leaves in EDU-treated plants (Table 2). Wang et al. (2007), however, found that EDU concentrations of 150 and 300 ppm had an insignificant effect on the above ground biomass of wheat and rice, although a decline in biomass was observed at the 450 ppm EDU treatment level.

5.2 EDU and Visible Injury

Ozone-induced visible injury on plants includes flecking, the appearance of having a water soaked area, interveinal stippling, chlorosis, bronzing and necrosis, all of which lead to early senescence of leaves. Young leaves are less susceptible to O_3 injury than are old leaves, because aging leaves contain lower levels of antioxidants than younger ones (Bisessar 1982). This suggests that EDU is more effective during the later stages of plant life than at early stages. Lee et al. (1981) also showed that senescence of red clover leaf discs was delayed when discs were floated on a EDU solution in the dark or under low intensity light. Kostka-Rick and Manning (1992) noted complete O_3 injury protection in EDU-treated radish plants (Table 3).

In Table 3, we provide a summary of experiments conducted to assess the response of O_3 on foliar injury when EDU treatments were used. EDU at 400 ppm completely protected against visible injury from O_3 exposure in soybean plants in Pakistan (Wahid et al. 2001). EDU, used as a low dose soil drench (15 ppm), provided complete protection from accelerated foliar senescence in potato that had been exposed for 11 day to 0.1 $\mu L\ L^{-1}$ O_3 for 5 h day^{-1} (Eckardt and Pell 1996). Kostka-Rick and Manning (1993b, c) showed similar foliar injury protection at a low EDU dose (100 mg L^{-1}), although toxicity symptoms occurred at higher dosages (300–800 mg L^{-1}). Postiglione and Fagnano (1995) used three O_3-sensitive plants (subterraneum clover, bean and tomato) and one O_3-tolerant plant (lettuce) to substantiate that EDU protection was not complete, (i.e., visible) injury was observed, though it was delayed and displayed a reduced intensity. Fumagalli et al. (1997) noted that the percent of O_3 injured leaves was significantly reduced in potted *Trifolium repens* plants that had been treated with 150 ppm EDU as a soil drench.

5.3 Role of EDU in Physiology and Photosynthetic Pigments of Plants

The physiological effects of EDU that are linked with its protective properties are still unclear (Blum et al. 2011). Previous studies showed that EDU mitigated O_3 effects through biochemical modifications in plants and not by biophysical changes (Bennett et al. 1978; Lee and Bennett 1982; Hassan et al. 1995). Stomatal conductance in EDU-treated plants that were exposed to ambient O_3 has been measured in only a few studies. In most such studies, the authors have reported no significant EDU effects on stomatal conductance (Bennett et al. 1978; Ainsworth et al. 1996; Hassan et al. 2007). Agrawal and Agrawal (1999) were first to provide evidence that EDU may act against O_3 exposure at the biophysical level by decreasing stomatal conductance. The percent closed stomata were 39.1, 45.7, 55.3 and 66.7, respectively, in control, EDU treated, O_3-exposed and EDU + O_3-exposed snap bean plants. Later, field study results disclosed the action of EDU on plant physiology (see Table 4). Recently, Wahid et al. (2012) found that sesame plants treated with 500 ppm EDU showed an increase of 52 % in stomatal conductance and a 61 % increase in net-photosynthesis rate, compared to non-EDU treated plants. Ozone is known to damage the thylakoid membrane in chloroplasts, which negatively affects

Table 3 Effects of EDU treatment on ozone-induced injury of plant species

Country/site	Species/cultivar	EDU application	EDU dose/ conc.	O_3 conc.	Application interval	Injury symptoms	Injury	Reference
Belgium	*Populus nigra* L. cv. Wolterson	Stem injection	0 mg/plant 1 mg/plant 2.5 mg/plant 4 mg/plant 0 mg/plant 1 mg/plant 2.5 mg/plant 4 mg/plant	150–300 ppb for 6 h	–	Injured leaves Injured leaf surface	18 % 3.5 % 1.7 % 0 7.0 % 0.4 % 0.1 % 0	Bortier et al. (2001)
Egypt Suburban Rural	*Raphanus sativus* L. *Brassica rapa* L. *Raphanus sativus* L. *Brassica rapa* L.	Soil drench	500 ppm/ 200 mL	54.8 ppb (6 h) 66.9 ppb (6 h)	10 days	Chlorotic spots	5 fold (↓) No injury 2 fold (↓) No injury	Hassan et al. (1995)
Suburban Rural	*Solanum tuberosum* L. Kara	Foliar spray	300 ppm/ 300 mL	78 ppb (10 h) 95.5 ppb (10 h)	10 days	Pinpoint brown dots followed by bronze lesions and necrotic spots	65 % (↓) 82 % (↓)	Hassan (2006)

(continued)

Table 3 (continued)

Country/site	Species/cultivar	EDU application	EDU dose/conc.	O_3 conc.	Application interval	Injury symptoms	Injury	Reference
Rural	*Trifolium alexandrinum* L. Messkawy	Soil drench	0 ppm/200 mL 50 ppm/200 mL 100 ppm/200 mL 150 ppm/200 mL 200 ppm/200 mL	88 ppb (8 h day^{-1})	10 days	Number of injured leaves (flecking)	1.98 % 1.56 % 1.02 % 0.45 % 0.42 %	Hassan et al. (2007)
Germany	*Phaseolus vulgaris* L. Lit	Soil drench	150 ppm/200 mL	–	14 days	Bronzing	Complete protection	Brunschon-Harti et al. (1995a)
Italy	*Populus* L. cv. I-214 and Eridano	Stem injection	0 ppm/0.5 mL 250 ppm/0.5 mL 1,000 ppm/1.0 mL	85 ppb for 8 h day^{-1}	10 days	No injury in I-214 clone Small interveinal small stipples, necrotic area	2.07 % 1.52 % 0.65 %	Ainsworth et al. (1996)
	Fraxinus excelsior L.	Gravitational infusion	0 ppm/13.3 mL/plant 300 ppm/13.3 mL/plant 450 ppm/8.9 mL/plant	150 ppb for 8 h day^{-1}, 21 days	–	Interveinal reddish stipples	13 % 3 % 2 %	Paoletti et al. (2007)

Maryland	*Phaseolus vulgaris* L. Bush Blue Lake 290	Soil drench	0 mg/pot, 100 mL/ plant 12.5 mg/ pot, 100 mL/ plant 25 mg/pot, 100 mL/ plant 50 mg/pot, 100 mL/ plant	458 ppb	–	Stippling, bifacial necrosis or marginal and tip burn	87 % 75 % 38 % 0 %	Lee and Bennett (1982)
	Trifolium pratense L. cv. Pennscott	–	0 μg/mL 125 μg/mL 250 μg/mL 500 μg/mL	303 ppb for 4 h	–	Stippling, bifacial necrosis or marginal and tip burning	83 % 73 % 22 % Complete protection	Lee et al. (1981)
Ontario	*Arachis hypogaea* L. USDA PI 268661 McRan (Laboratory study) USDA PI 268661 McRan (Field study)	Foliar spray	1 g/L	219 ppb/7 h day^{-1} for 4 d 219 ppb	14 days	White flecks, bronzing. chlorosis only in USDA PI 268661 Foliar bronzing and chlorosis	87.7 % (↓) 75.0 % (↓) 91.4 % (↓) No injury	Ensing et al. (1985)

(continued)

Table 3 (continued)

Country/site	Species/cultivar	EDU application	EDU dose/ conc.	O_3 conc.	Application interval	Injury symptoms	Injury	Reference
USA	*Raphanus sativus* L. cv. Cherry Belle	Soil drench	150 ppm/ 60 mL/ plant	70 ppb/7 h day^{-1}, 5 d	7 days	Leaf margin necrosis and leaf deformation	Complete protection	Kostka-Rick and Manning (1992)
	Phaseolus vulgaris L. Bush Blue Lake 290	Soil drench	0 ppm/ 100 mL 500 ppm/ 100 mL	300 ppb for 3 h	–	Necrosis	45 % 10 %	Lee et al. (1997)
	Rudbeckia laciniata L.	Foliar spray	0 ppm 200 ppm 400 ppm 600 ppm	32–38 ppb (12 h)	7 days	Leaves injured	13.9 % 13.2 % 11.1 % 9.2 %	Szantoi et al. (2009)
Wilmington, DE USA	*Phaseolus vulgaris* L. Pinto 111	Root application	0 mg/ 20 mL 4 mg/ 20 mL	120 ppm for 150 min	–	–	88 % 8 %	Carnahan et al. (1978)

Wooster, OH, USA	*Phaseolus vulgaris* L.	Runoff on leaf surface	0 µg/g	0.0 ppm	–	Number of leaf injured (Severe bleaching and bifacial necrosis)	8	Weidensaul (1980)
			500 µg/g	0.10 ppm	–		8	
			1,000 µg/g	0.25 ppm	–		8	
			5,000 µg/g	0.40 ppm	–		8	
			0 µg/g	0.50 ppm	–		8.2	
			500 µg/g	0.75 ppm	–		8	
			1,000 µg/g				8	
			5,000 µg/g				8	
			0 µg/g				12.4	
			500 µg/g				11.7	
			1,000 µg/g				10.5	
			5,000 µg/g				9.9	
			0 µg/g				19.5	
			500 µg/g				16.2	
			1,000 µg/g				15.2	
			5,000 µg/g				15.4	
			0 µg/g				26.6	
			500 µg/g				22.8	
			1,000 µg/g				21.3	
			5,000 µg/g				14.6	
			0 µg/g				26.3	
			500 µg/g				24.0	
			1,000 µg/g				23.6	
			5,000 µg/g				19.2	

(↓) = reduction

the light quenching capacity of chlorophyll. EDU application improved fluorescence kinetics in O_3-exposed plants of *Beta vulgaris* (Tiwari and Agrawal 2009), *Triticum aestivum* (Singh and Agrawal 2009) and *Trifolium repens* (Singh et al. 2010d) (Table 4). A study on the photosynthetic capacity of ash trees revealed a slight effect of EDU in preventing O_3-induced inactivation of photosynthetic reaction centers (Contran et al. 2009).

Photosynthetic pigments are the basic entities of the plant photosynthetic machinery, and EDU is effective in maintaining high levels of photosynthetic pigments. Lee and Chen (1982) reported that non-EDU-treated leaf discs of tobacco lost more than half of their original chlorophyll after 10 days, whereas discs treated with 1×10^{-3} M EDU lost only 10 % of the initial chlorophyll. Whitaker et al. (1990), however, found that EDU treatment did not alter leaf chlorophyll or carotenoid contents, but a reduction of 14 % was observed when plants were exposed to O_3 alone. Potato plants treated with 45 and 75 mg EDU L^{-1} had higher chlorophyll content, and the chlorophyll was retained for a longer period than in untreated plants (Eckardt and Pell 1996). One main reason that EDU provides protection was that it enhances retention of chlorophyll for longer time (Lee et al. 1997). In Table 5, we summarized results of investigations that have been conducted to evaluate the effects on photosynthetic pigments from O_3 exposure and EDU treatment.

5.4 *EDU Protection in Relation to Antioxidants*

EDU does not act as an antioxidant itself, but helps to maintain higher levels of cellular antioxidants during O_3 stress in *Phaseolus vulgaris* (Lee et al. 1997). Superoxide dismutase (SOD) activity is normally associated with O_3 tolerance. Lee and Bennett (1982) found a significant induction of SOD and catalase (CAT) activities when snap beans were treated with EDU. Pitcher et al. (1992) refuted the hypothesis that EDU's mode of action works by increasing SOD activity. There was no EDU associated increases in Cu/Zn-SOD or Mn-SOD activities in plants exposed to O_3. Lee et al. (1997) did not observe significant differences in activities of ascorbate peroxidase (APX), guaicol peroxidase (GPX) and SOD in O_3 exposed snap bean leaves that were treated with EDU as compared to untreated plants. However, EDU-treated plants maintained a higher glutathione reductase (GR) activity. Batini et al. (1995) hypothesized that the protection mechanism against O_3 that had been exerted by EDU was caused by the stimulation of only the APX detoxifying system involved in scavenging hydrogen peroxide molecules. Singh et al. (2010b) found more significant reductions in POX and SOD activities in leaves of EDU-treated mungbean plants than in non EDU-treated ones. A compilation of experiments conducted to assess the effects of EDU on antioxidant enzymes under O_3 treatment is given in Table 6.

Among non-enzymatic antioxidants, apoplastic ascorbic acid acts as a 'first line of defense' against O_3 damage (Chameides 1989). Hence, ozone tolerance is

Table 4 Effects of EDU treatment on Fv/Fm ratio, photosynthetic rate and stomatal conductance of plants grown under ozone exposure

Country/ site	Species/cultivar	EDU application	EDU dose/ conc.	O_3 conc.	Application time/ interval	Fv/Fm ratio	Photosynthetic rate	Stomatal conductance	Reference
Egypt Rural	*Raphanus sativus* L. *Brassica rapa* L.	Soil drench	500 ppm/ 200 mL	66.9 ppb (6 h)	10 days	–	NS NS	NS NS	Hassan et al. (1995)
India	*Phaseolus vulgaris* L. Bush Blue Lake 290	Soil drench	500 ppm/ 100 mL	305 ppb for 3 h	48 h prior to fumigation	–	77.7 % (↑)	27.6 % (↓)	Agrawal and Agrawal (1999)
Urban	*Triticum aestivum* L. HD 2329 HUW 234 HUW 468	Soil drench	500 ppm/ 250 mL	29.2 ppb (6 h)	7 days	– – –	– – –	2.8 % (↓) 6.2 % (↓) 14.8 % (↓)	Agrawal et al. (2004)
Suburban	*Beta vulgaris* L. Allgreen	Soil drench	300 ppm/ 200 mL	52.1–73.2 ppb (8 h)	10 days	1.7 % (↑)	–	–	Tiwari and Agrawal (2009)
Suburban	*Triticum aestivum* L. HUW234 HUW468 HUW510 PBW343 Sonalika	Soil drench	400 ppm/ 100 mL	35.3–54.2 ppb (8 h)	12 days	14.5 % (↑) 6.1 % (↑) 5.6 % (↑) NS NS	20.7 % (↑) 27.2 % (↑) 22.1 % (↑) NS NS	16.0 % (↑) 16.9 % (↑) 13.7 % (↑) NS NS	Singh and Agrawal (2009)

(continued)

Table 4 (continued)

Country/ site	Species/cultivar	EDU application	EDU dose/ conc.	O_3 conc.	Application time/ interval	Fv/Fm ratio	Photosynthetic rate	Stomatal conductance	Reference
Rural	*Vigna radiata* L. Malviya Janpriya	Soil drench	400 ppm/ 100 mL	52.9–64.5 ppb (12 h)	10 days	–	31.6 % (↑)	31.2 % (↑)	Singh et al. (2010b)
	Trifolium repens L. Bundel Vardan	Soil drench	150 ppm/ 100 mL 300 ppm/ 100 mL 150 ppm/ 100 mL 300 ppm/ 100 mL	30.3–46.6 ppb (12 h)	10 days	NS NS 5.4 % (↑) 5.4 % (↑)	– – – –	– – – –	Singh et al. (2010d)
Pakistan Suburban	*Sesamum indicum* L. Ts-3 Til 93 S-17	Soil drench	125 ppm/ 100–400 mL 250 ppm/ 100–400 mL 375 ppm/ 100–400 mL 500 ppm/ 100–400 mL	Seasonal mean91 ppb (10 h)	7days	– – – –	NS 25–35 % (↑) 47–52 % (↑) 56–61 % (↑)	NS 13–16 % (↑) 33–39 % (↑) 47–52 % (↑)	Wahid et al. (2012)

(↑) = increase, (↓) = decrease, *NS* not significant, *Fv* variable fluorescence, *Fm* maximum fluorescence

Table 5 Effects of EDU treatment on photosynthetic pigments, protein content, MDA and ascorbic acid contents of plants grown under ozone exposure

Country/ site	Species/ cultivar	EDU application	EDU doses/ conc.	O_3 conc.	Application interval	Chlorophyll content	Carotenoid content	Protein content	MDA content	Ascorbic acid content	Reference
India Urban	*Triticum aestivum* L. HD 2329 HUW 234 HUW 468	Soil drench	500 ppm/ 250 mL	29.2 ppb (6 h)	7 days	(↑) (↑) (↑)	(↑) (↑) (↑)	– – –	– – –	(↑) (↑) (↑)	Agrawal et al. (2004)
Suburban	*Vigna radiata* L. Malviya Jyoti	Soil drench	500 ppm/ 500 mL	32.6–35.2 ppb (8 h)	7 days	12.8 % (↑)	(↑)	9.8 % (↑)	–	13.8 % (↑)	Agrawal et al. (2005)
Suburban	*Beta vulgaris* L. Allgreen	Soil drench	300 ppm/ 200 mL	52.1–73.2 ppb (8 h)	10 days	17.5 % (↑)	28 % (↑)	20.4 % (↑)	37.4 % (↓)	39.8 % (↑)	Tiwari and Agrawal (2009)
Suburban	*Triticum aestivum* L. HUW234 HUW468 HUW510 PBW343 Sonalika	Soil drench	400 ppm/ 100 mL	35.3–54.2 ppb (8 h)	12 days	19.1 % (↑) 22.6 % (↑) 18.9 % (↑) NS NS	22.5 % (↑) 28.3 % (↑) 17.9 % (↑) 26.5 % (↑) 27.8 % (↑)	11.8 % (↑) 14.4 % (↑) 28.15 (↑) 13.9 % (↑) 2.0 % (↑)	13.6 % (↓) 16.2 % (↓) 6.6 % (↓) NS 18.4 % (↓)	NS 17.5 % (↑) 11.5 % (↑) NS NS	Singh and Agrawal (2009)

(continued)

Table 5 (continued)

Country/ site	Species/ cultivar	EDU application	EDU doses/ conc.	O_3 conc.	Application interval	Chlorophyll content	Carotenoid content	Protein content	MDA content	Ascorbic acid content	Reference
Suburban	*Triticum aestivum* L. HUW468	Soil drench	200 ppm/ 100 mL 300 ppm/ 100 mL 400 ppm/ 100 mL 500 ppm/ 100 mL	27.7–59.1 ppb (12 h)	10 days	13.8 % (↑) 11.2 % (↑) 25.2 % (↑) 7.2 % (↑)	29.5 % (↑) 2.9 % (↑) 31.6 % (↑) 14.8 % (↑)	–	–	–	Singh and Agrawal (2010)
Rural	*Vigna radiata* L. Malviya Janpriya	Soil drench	400 ppm/ 100 mL	52.9–64.5 ppb (12 h)	10 days	(↑)	(↑)	(↑)	17.7 % (↓)	60.5 % (↑)	Singh et al. (2010b)
Saudi Arab KSU KFS	*Vicia faba* L. Lara	Soil drench	250 ppm	21.2 ppb 62.7 ppb	12 days	18.6 % (↑) 13.6 % (↑)	14.1 % (↑) 10.7 % (↑)	– –	– –	– –	Al–Qurainy (2008)
USA	*Phaseolus vulgaris* L. Bush Blue Lake 290	Soil drench	500 ppm/ 100 mL	300 ppb for 3 h		144.4 % (↑)	–	–	–	–	Lee et al. (1997)

(↑) = increase, (↓) = decrease, *NS* not significant, *MDA* malondialdehyde

Table 6 Effects of EDU treatment on some antioxidant enzymes of plants grown under ozone exposure

Country/ site	Species/cultivar	EDU application	EDU doses/ conc.	O_3 conc.	Application time/interval	Parameters	Changes	Reference
Germany	*Phaseolus vulgaris* L. Lit	Soil drench	150 ppm/ 200 mL	0.98–31.5 ppb	14 days	POX CAT APX	−0.42 fold +1.47 fold NS	Brunschon-Harti et al. (1995b)
India Suburban	*Triticum aestivum* L. HUW234 HUW468 HUW510 PBW343 Sonalika	Soil drench	400 ppm/ 100 mL	35.3–54.2 ppb (8 h)	12 days	SOD POX APX SOD POX APX SOD POX APX SOD POX APX SOD POX APX	NS +1.64 fold +1.84 fold +1.21 fold +2.32 fold +2.21 fold +1.18 fold +2.10 fold +2.73 fold NS NS NS NS NS NS	Singh et al. (2009b)

(continued)

Table 6 (continued)

Country/ site	Species/cultivar	EDU application	EDU doses/ conc.	O_3 conc.	Application time/interval	Parameters	Changes	Reference
Suburban	*Beta vulgaris* L. Allgreen	Soil drench	300 ppm/ 200 mL	52.1–73.2 ppb	10 days	POX	−0.77 fold	Tiwari and Agrawal (2009)
Rural	*Vigna radiata* L. Malviya Janpriya	Soil drench	400 ppm/ 100 mL	52.9–64.5 ppb (12 h)	10 days	SOD POX	−0.46 fold −0.47 fold	Singh et al. (2010b)
Maryland	*Phaseolus vulgaris* L. Bush Blue Lake 290	Soil drench	25 mg/pot 50 mg/pot 100 mg/pot in 100 mL	457 ppb for 4 h	24 h before O_3 treatment	SOD CAT POX	+1.07 fold +2.31 fold +1.32 Fold +1.17 Fold +2.04 Fold +1.64 fold +1.07 fold +1.97 fold +5.84 fold	Lee and Bennett (1982)

New Jersey	*Phaseolus vulgaris* L. Bush Blue Lake 290	Soil drench	0.3 mg/mL	0.3 ppm for 6 h	24 h before O_3 treatment	SOD	−0.95 fold	Pitcher et al. (1992)
U.S.A.	*Phaseolus vulgaris* L. Bush Blue Lake 290	Soil drench	500 ppm/ 100 mL	300 ppb for 3 h		GR APX GPX CAT SOD	+1.36 fold No change No change NS NS	Lee et al. (1997)

+ = increase, − = decrease

directly correlated to increased apoplastic ascorbic acid in *P. major* population (Zheng et al. 2000), snap bean ecotypes (Burkey 1999; Burkey et al. 2003), soybean cultivars (Robinson and Britz 2000, 2001) and *Sedum album* (Castillo and Greppin 1988). Various studies have revealed that EDU plays a role in maintenance/ synthesis of the ascorbic acid pool in plants. Significant increase in the ratio of ascorbic acid (AA)/dehydroascorbic acid (DHA) in EDU-treated plants has been shown by Brunschon-Harti et al. (1995b). However, Gillespie et al. (1998) did not find any significant effect of EDU on the AA and DHA contents of snap bean plants. Singh et al. (2010b) reported a significantly higher content of ascorbic acid in mungbean that had been treated with EDU. Higher ascorbic acid content in foliage is commonly observed to occur after EDU treatment (Table 5).

To understand the EDU-induced O_3 tolerance and the role of glutathione (GSH) in snap bean, Lee et al. (1997) used an HPLC technique to study the effect of EDU. They found that total glutathione and GSH concentrations decreased significantly in O_3 fumigated plants (no EDU + O_3), but GSSG concentrations increased *vis-a-vis* a decrease in the GSH/GSSG ratio, compared to controls (no O_3). Pretreatment with EDU significantly increased the levels of total glutathione and GSH, and reduced the level of GSSG, in comparison to control snap bean plants. Higher concentrations of total glutathione and GSH and a lower GSSG reserve were observed in EDU-treated plants after O_3 exposure (EDU + O_3) vs. control plants (no EDU + O_3). This suggested that EDU-treated plants that were under O_3 stress showed no reduction in glutathione reductase (GR) activity. Lee et al. (1997) suggested that EDU protection against O_3 damage may result from maintenance of GR and GSH levels during O_3 exposure. Hassan (2006) used the same technique to determine the glutathione concentration in potato leaves. EDU-treated plants grown under O_3 exposure had a higher GSH content, but lower GSSG and total glutathione levels, compared to plants exposed to O_3 without EDU treatment. Generally, plants treated with EDU have a higher GSH/GSSG ratio than do the controls.

Polyamines are known as stabilizing factors of biomembranes and macromolecules and are also free radical scavengers (Drolet et al. 1986), either directly or via conjugation with other molecules such as free ferulic and caffeic acids (Didyk and Blum 2011). Bors et al. (1989) suggested that polyamines conjugate with hydroxycinnamic acids and play a role in protecting against the accumulation of O_3-triggered reactive oxygen species. Polyamine content in fully expanded leaves of snap bean treated with EDU was compared with the control (-EDU) leaves before and after O_3 exposure. It was reported that EDU did not alter the polyamine composition of leaves, although O_3 alone (-EDU) induced significant increases in total polyamines (Lee et al. 1992).

5.5 Effects of EDU on Soluble Protein, MDA (Malondialdehyde) Content and Foliar Lipids

Brunschon-Harti et al. (1995b) showed that the soluble protein content increased following EDU treatment in snap bean plants, while increasing O_3 dose did not cause any change in total soluble protein in EDU-treated plants. Studies have revealed that an increased O_3 dose increased the MDA content in EDU-treated plants, thus reflecting more damage to plasma membranes. Most study results support the view that the MDA content is increased from EDU treatment, although there are exceptions (Table 5). Whitaker et al. (1990) found that pretreatment with EDU conferred protection against O_3-induced losses of glycerolipids. Leaves of untreated snap bean plants lost approximately 50 % of both the galactolipids (GL) and phospholipids (PL), while EDU-treated plants showed no significant loss of foliar GL and PL. In controls (no EDU), sterylglycosides (SG) showed an incremental increase, while EDU-treated plants showed a small increase in SG.

5.6 Effects of EDU on Carbohydrates

Increments in sucrose and other soluble sugars in bean leaves, 48 h after EDU treatment was correlated with development of resistance against O_3 (Lee et al. 1981). Miller et al. (1994) reported higher starch levels in EDU-treated O_3-exposed snap bean plants, whereas foliar starch levels declined under O_3 exposure without EDU treatment, compared to plants exposed to charcoal filtered (CF) air. Singh et al. (2010c) observed that all three test cultivars of blackgram (barkha, shekhar and TU-94-2) showed a significant decrease in reducing sugar content, and a respective increase in total soluble sugar content in seeds of plants treated with EDU. In contrast, starch content increased significantly only in seeds of EDU-treated black gram cultivar TU-94-2 as compared to non-EDU treated ones. Al-Qurainy (2008) studied broad bean plants grown without EDU at O_3 concentrations of 21.2 and 62.7 ppb in ambient air, respectively at King Saud University and King Fahad Street, displayed reduced soluble, insoluble and total leaf carbohydrates as they aged. In contrast, plants treated with EDU at 250 mg L^{-1}showed increments in all forms of carbohydrates with increasing age, compared to non EDU-treated plants.

6 The Role of EDU in Assessing Yield Losses

O_3-related yield losses are a serious and growing concern throughout the world. Different approaches have been utilized to estimate the level of economic losses experienced from O_3 exposure. EDU is a widely used biomonitoring tool that is commonly utilized to assess plant yield loss from O_3 exposure. EDU successfully protects a wide variety of plants from O_3. EDU has also been used to screen for O_3 sensitive and tolerant/resistant cultivars, and to estimate the extent of damage caused by O_3. Such information is useful for selecting cultivars to grow in areas that experience high O_3 levels. Yield loss assessments are performed by comparing the yield estimates of EDU-treated and untreated plants in O_3 polluted areas of the USA (Ensing et al. 1985; Smith et al. 1987), Asia (Wahid et al. 2001; Agrawal et al. 2004, 2005; Tiwari et al. 2005; Wang et al. 2007; Singh and Agrawal 2009; Singh et al. 2010b, c), Africa (Hassan et al. 1995; Hassan 2006) and Europe (Ribas and Penuelas 2000; Brunschon-Harti et al. 1995a; Pleijel et al. 1999). Yield increments after the application of EDU onto O_3 exposed plants have been reported for onion (Wukasch and Hofstra 1977), navy bean (Hofstra et al. 1978; Temple and Bisessar 1979; Toivonen et al. 1982), tomato (Legassicke and Ormrod 1981), potato (Bisessar 1982; Clarke et al. 1990; Hassan 2006), tobacco (Bisessar and Palmer 1984), watermelon (Fieldhouse 1978), peanut (Ensing et al. 1985), radish (Kostka-Rick and Manning 1992; Hassan et al. 1995), carrot (Tiwari and Agrawal 2010), bush bean (Kostka-Rick and Manning 1993a, c), snap bean (Vandermeiren et al. 1995), mungbean (Agrawal et al. 2005; Singh et al. 2010b), soybean (Wahid et al. 2001), wheat (Agrawal et al. 2004; Tiwari et al. 2005; Wang et al. 2007; Singh and Agrawal 2009) and black gram (Singh et al. 2010c).

Applying EDU has increased the number of seeds per plant and total seed weight per plant for two O_3-sensitive cultivars of soybean, while insignificant effects were observed in the O_3-tolerant lines for these parameters (Damicone 1985). No significant difference in seed size was observed vs. controls in soybean cultivars that were treated with EDU and then exposed to O_3 (Smith et al. 1987). EDU treatment has led to increased seed size in wheat (Agrawal et al. 2004; Singh and Agrawal 2009), and mungbean (Agrawal et al. 2005). Wahid et al. (2001) reported significantly higher seed weight/plant, number of seeds/pod and number of pods/plant for *Glycine max* under EDU treatment at all experimental sites (suburban, rural and rural roadside) in Pakistan that experienced higher O_3 concentrations during pre-monsoon and post-monsoon experiments. Significantly higher tuber weight and number of tubers were reported in EDU-treated potato plants, compared to EDU-untreated plants at both rural and suburban sites in Egypt in areas experiencing high O_3 concentrations (Hassan 2006). In Table 7, we have summarized results of experiments, in which the effect of O_3 on yield was assessed, along with the mitigating effects of having used EDU.

EDU has been widely and successfully used to protect against the effects of O_3, as a research tool to assess plant responses to O_3 stress, and to estimate crop/plant yield losses. The advantages and disadvantages of using EDU for these purposes are stated below:

Table 7 Effects of EDU treatment on yield of plants grown under ozone exposure

Country/ site	Species/cultivar	EDU application	EDU dose/conc.	O_3 conc.	Application interval	Yield	Reference
China	*Triticum aestivum* L. *Oryza sativa* L.	Foliar spray	150 ppm 300 ppm 450 ppm 150 ppm 300 ppm 450 ppm	Frequently exceeding 40 ppb	7 days	3.4 % (↑) 12.7 % (↑) 7.1 % (↑) NS NS NS	Wang et al. (2007)
Egypt Suburban Rural	*Raphanus sativus* L. *Brassica rapa* L. *Raphanus sativus* L. *Brassica rapa* L.	Soil drench	500 ppm/200 mL	54.8 ppb (6 h) 66.9 ppb (6 h)	10 days	33.3 % (↑) 4.3 % (↑) 36.2 % (↑) 20.9 % (↑)	Hassan et al. (1995)
Egypt Suburban Rural	*Solanum tuberosum* L. Kara	Foliar spray	300 ppm/300 mL	78 ppb (10 h) 95.5 ppb (10 h)	10 days	30.5 % (↑) 31.8 % (↑)	Hassan (2006)
Germany	*Phaseolus vulgaris* L. Lit	Soil drench	150 ppm/200 mL	0.98–31.5 ppb	14 days	30–65 % (↑)	Brunschon-Harti et al. (1995a)
India Urban	*Triticum aestivum* L. HD 2329 HUW 234 HUW 468	Soil drench	500 ppm/250 mL	29.2 ppb (6 h)	7 days	22 % (↑) 27 % (↑) 36.3 % (↑)	Agrawal et al. (2004)
Suburban	*Vigna radiata* L. Malviya Jyoti	Soil drench	500 ppm/500 mL	32.6–35.2 ppb (8 h)	7 days	32.2 % (↑)	Agrawal et al. (2005)

(continued)

Table 7 (continued)

Country/ site	Species/cultivar	EDU application	EDU dose/conc.	O_3 conc.	Application interval	Yield	Reference
Suburban	*Triticum aestivum* L. M 234 M 533	Soil drench	150 ppm/200 mL 300 ppm/200 mL 450 ppm/200 mL 150 ppm/200 mL 300 ppm/200 mL 450 ppm/200 mL	Often exceeding 40 ppb (8 h)	10 days	24.8 % (↑) 66.9 % (↑) 66.8 % (↑) 18.8 % (↑) 19.1 % (↑) 20.5 % (↑)	Tiwari et al. (2005)
Suburban	*Triticum aestivum* L. HUW234 HUW468 HUW510 PBW343 Sonalika	Soil drench	400 ppm/100 mL	35.3–54.2 ppb (8 h)	12 days	11.2 % (↑) 25.8 % (↑) 20.5 % (↑) 1.9 % (↑) 10.2 % (↑)	Singh and Agrawal (2009)
Suburban	*Glycine max* L. Pusa 9712 Pusa 9814	Soil drench	400 ppm/200 mL	NFCs NFCs + 20 ppb NFCs NFCs + 20 ppb	10 days	29.8 % (↑) 33.0 % (↑) 28.2 % (↑) 29.0 % (↑)	Singh and Agrawal (2011)

Rural	*Vigna radiata* L. Malviya Janpriya	Soil drench	400 ppm/100 mL	52.9–64.5 ppb (12 h)	10 days	32.3 % (↑)	Singh et al. (2010b)
Suburban	*Vigna mungo* L. cv. Barkha Shekhar TU-94-2	Soil drench	400 ppm/100 mL	41.3–59.9 ppb (12 h)	10 days	36.4 % (↑) 35.6 % (↑) NS	Singh et al. (2010c)
Ontario	*Arachis hypogaea* L. PI 268661	Foliar spray	1,000 ppm	220 ppb (7 h day^{-1}) for 4 d	14 days	24.0 % (↑)	Ensing et al. (1985)
Pakistan Punjab Post-monsoon Rural roadside Rural Suburban Pre-monsoon Rural roadside Rural Suburban	*Glycine max* L. NARC-1	Soil drench	400 ppm/600 mL	48 ppb (6 h) 48 ppb (6 h) 40 ppb (6 h) 70 ppb (6 h) 75 ppb (6 h) 63 ppb (6 h)	10 days	170 % (↑) 94 % (↑) 47 % (↑) 284.6 % (↑) 181.8 % (↑) 112.8 % (↑)	Wahid et al. (2001)
Spain	*Phaseolus vulgaris* L.	Soil drench	Increasing dose of 100, 150, 200 and 250 ppm/200 mL	10,000 ppb h (AOT40)	12 days	58.4 % (↑)	Ribas and Penuelas (2000)
Sweden	*Raphanus sativus* L. Cherry Belle	Soil drench	200 ppm/100 mL	31 ppb (24 h)	14 days	32 % (↑)	Pleijel et al. (1999)

(↑) = increase, (↓) = decrease, *NS* not significant

6.1 Advantages

1. No chambers or costly investments like FACE (Free Air Carbon dioxide Enrichment) are required. The use of EDU requires only ambient conditions; no modifications of microclimate are needed.
2. Plant numbers and plot sizes can vary according to the requirements of the experiment, which facilitates having many replications.
3. Low technical input is required to utilize EDU, whose utilization is comparatively simple and easy to execute.

6.2 Disadvantages

1. Ozone dose-response studies cannot be performed, unless coupled with OTCs (open top chambers).
2. Ambient O_3 concentrations and environmental variables need careful monitoring.
3. Repeated applications of EDU can cause phytotoxicity, especially under dry soil conditions; hence, detailed plant toxicological studies are needed before starting field experiments.
4. High cost and commercial non availability of EDU has led it to be used mainly in monitoring experiments; the commercial scale use of EDU as a crop protectant has not yet occurred.
5. The use of EDU requires extensive labor and time for application.

7 Summary

Urbanization, industrialization and unsustainable utilization of natural resources have made tropospheric ozone (O_3) one of the world's most significant air pollutants. Past studies reveal that O_3 is a phytotoxic air pollutant that causes or enhances food insecurity across the globe. Plant sensitivity, tolerance and resistance to O_3 involve a wide array of responses that range from growth to the physiological, biochemical and molecular. Although plants have an array of defense systems to combat oxidative stress from O_3 exposure, they still suffer sizable yield reductions. In recent years, the ground-level O_3 concentrations to which crop plants have been exposed have caused yield loses that are economically damaging. Several types of chemicals have been applied or used to mitigate the effects produced by O_3 on plants. These include agrochemicals (fungicides, insecticides, plant growth regulators), natural antioxidants, and others. Such treatments have been effective to one degree to another, in ameliorating O_3-generated stress in plants. Ethylene diurea (EDU) has been the most effective protectant used and has also served as a monitoring agent for assessing plant yield losses from O_3 exposure. In this review, we summarize the data on how EDU has been used, the treatment methods tested, and application doses found to be both protective and toxic in plants. We have also summarized data that address the nature and modes of action (biophysical and biochemical) of EDU.

In general, the literature discloses that EDU is effective in reducing ozone damage to plants, and indicates that EDU should be more widely used on O_3 sensitive plants as a tool for biomonitoring of O_3 concentrations. Biomonitoring studies that utilize EDU are very useful for rural and remote areas and in developing countries where O_3 monitoring is constrained from unavailability of electricity. The mechanism(s) by which EDU prevents O_3 toxicity in plants is still not completely known. EDU possesses great utility for screening plant sensitivity under field conditions in areas that experience high O_3 concentrations, because EDU prevents O_3 toxicity only in O_3 sensitive plants. Ozone-resistant plants do not respond positively to EDU applications. However, EDU application dose and frequency must be standardized before it can be effectively and widely used for screening O_3 sensitivity in plants. EDU acts primarily by enhancing biochemical plant defense and delaying O_3-induced senescence, thereby reducing chlorophyll loss, and maintaining physiological efficiency and primary metabolites; these actions enhance growth, biomass and yield of plants.

We believe that future studies are needed to better address the EDU dose-response relationship for many plant species, and to screen for new cultivars that can resist O_3 stress. Although some research on the physiological and biochemical mechanisms of action of EDU have been performed, the new ‘omics’ tools have not been utilized to evaluate EDUs mechanism of action. Such data are needed, as is gene expression and proteome profiling studies on EDU-treated and -untreated plants.

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