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Review

The possible effect of the bioaccumulation of disinfectant by-products on crops irrigated with treated wastewater

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The shortage of potable water for irrigation of food crops in semi-arid developing countries led to the use of alternative sources of water. Wastewater is gaining importance for productive use in agriculture throughout the world. A widely used and efficient method to reduce the occurrence of waterborne diseases in numerous wastewater plants is water chlorination. In early 1970s, some volatile halogenated organic compounds such as chloroform were identified in chlorinated surface waters containing high levels of natural organic material. Generally, the trihalomethanes (THMs), including chloroform, bromodicholoromethane, dibromochloromethane and bromoform were the most prevalent in chlorinated surface water. Predominant research studies focused on the carcinogenic and mutagenic properties of these compounds in treated wastewater. But little attention was paid to how these compounds in treated wastewater could affect crop performance in agriculture, physiological changes amongst crop varieties and the build-up of these organic compounds in edible plant tissues with persistent use of treated waste-water. A probable reason for this was the absence of the practice of wastewater irrigation in food crop agriculture in the past. Current knowledge on the trihalomethanes and possible plant interactions with this group of volatile organic compounds are assessed in this review.

Key words: Antioxidants, biomagnification, chlorophyll, metabolites, oxidative-stress, photosynthesis, phytotoxicity, seed germination, trihalomethanes.

INTRODUCTION

It was estimated in 1995 that about 2.3 billion people (41% of the world's population at that time) resided in river basins considered to be water stressed and this value was predicted to increase to 3.5 billion by 2025 (48 % of the expected world population) (World Resources Institute, 2000). This necessitated the need to implement other strategies to ease the pressures of demand on potable water. Hence, wastewater use in agriculture became a viable economic alternative (Anderson, 2003). In general, municipal wastewater is made up of domestic wastewater, industrial wastewater, storm water and by groundwater seepage entering the municipal sewage network (Saqer, 1996). Wastewaters of municipal and industrial origin are used to irrigate a wide variety of crops and landscapes across the world (Hamilton et al., 2005). Obstacles in the definition of wastewater and the lack of data on this subject have made it complex to arrive at a strong figure for global wastewater reuse for irrigation. Nevertheless, attempts were made and probably the best known estimate in 2001 was 20 million hectares of land which were irrigated with wastewater partially diluted or undiluted (Future Harvest, 2001). There have been several outstanding reviews on various aspects of wastewater irrigation, including health impacts and risks (Blumenthal and Peasey, 2002; Toze, 2006), the environmental fate of organics (Müller et al., 2007), management of salts (Rengasamy, 2006) and public perceptions (Hartley, 2003; Po and Nancarrow, 2004). Inventories of wastewater use in particular regions have been conducted (Angelakis et. al., 2001; Bixio et al., 2005; Radcliffe, 2004), but a broad overview of how

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many commonly found organic by-products in treated wastewater could affect agricultural productivity with persistent use in irrigation is lacking. This review will attempt to fill a small gap in this novel field with special emphasis on a group of volatile organic compounds formed from the chlorination of water known as the trihalomethanes (THM's).

It is generally accepted that the reaction between chlorine and humic substances, a major component of natural organic matter, is responsible for the production of organo-chlorine compounds during drinking-water treatment. Humic and fulvic acids show a high reactivity towards chlorine and constitute 50 - 90% of the total dissolved oxygen content in river and lake waters (Thurman, 1985). Most chlorine disinfectant by-products (DBP's) are formed through oxidation and substitution reactions. THMs have the general formula CHX_3, where X can be Cl^- or Br. Chloroform may be produced through a series of reactions with functional groups of humic substances (Johnson and Jensen, 1986).

Many treated waters contain not only chlorinated but also brominated compounds, such as bromoform. These compounds form because aqueous chlorine converts bromide in the water to hypobromous acid. The bromine can then react with the organic matter in the same way as hypochlorous acid to form various bromochlorinated DBPs (Gordon et al., 1997). The formation of organo-chlorine and organo-bromine compounds during drinking-water treatment is a cause of health concern in many countries (Reckhow et al., 1990). Chlorine dose is a factor affecting the type and concentration of DBPs formed. The THM level rises with increasing chlorine dose (Kavanaugh et al., 1980). Most investigators found a linear relationship between chlorine consumption and THM production, with an order of reaction greater than or equal to unity (Kavanaugh et al., 1980; Trussell and Umphres, 1978). As a class, the THMs are generally the most prevalent by-products of drinking-water disinfection by chlorine.

A variety of non-neoplastic toxic effects have been associated with short-term and long-term exposure of experimental animals to high doses of THMs (ICPS, 1994). The four most common THMs chloroform, bromodichloromethane, dibromochloromethane and bromoform has been shown to be carcinogenic to rodents in high-dose chronic studies. Chloroform is generally the predominant THM in chlorinated water and is also the most extensively studied chemical of this class because the World Health Organization (WHO) recently published an Environmental Health Criteria monograph on chloroform (IPCS, 1994).

The THMs are volatile liquids at room temperature; therefore, as these chemicals vaporize during water usage (e.g., showering), inhalation becomes an important exposure route in addition to ingestion (Kavanaugh et al., 1980). Perhaps to a greater extent than with other chemicals in this class, bromodichloromethane appears to reach a variety of target tissues where it can be readily metabolized to several intermediates, leading to adverse effects in experimental animals (Keegan et al., 1998). Vogt et al. (1979) reported that chloroform could be measured in the blood, brain, liver, kidneys and fat of rats to which sodium hypochlorite was administered. In a corn oil gavage study of single-dose chloroform effects, an increase in renal cell proliferation was observed at doses as low as 10 mg kg^{-1} of body weight in male Osborne-Mendel rats and 90 mg kg^{-1} of body weight in male F344 rats (Templin et al., 1996). Keegan et al. (1998) determined the lowest-observed-adverse-effect level (LOAEL) and no-observed-adverse-effect level (NOAEL) for the induction of acute hepatotoxicity following oral administration of chloroform in an aqueous vehicle to male F344 rats. The acute oral lethality of the brominated THMs has been determined in ICR Swiss mice (Bowman et al., 1978) and Sprague-Dawley rats (Chu et al., 1980).

Hewitt et al. (1983) gave single doses of bromodichloromethane to male Sprague-Dawley rats by corn oil gavage and found doses of 1980 mg kg^{-1} of body weight and above to be lethal. Thornton-Manning et al. (1994) administered five consecutive daily bromodichloromethane doses to female F344 rats and female C57BL/6J mice by aqueous gavage and found that bromodichloromethane is both hepatotoxic and nephrotoxic to female rats. A dibromochloromethane dose of 500 mg kg^{-1} of body weight produced ataxia, sedation and anesthesia in mice (Bowman et al., 1978). Depressed immune function was also observed in both sexes of CD-1 mice gavaged with dibromochloromethane in an aqueous vehicle given at doses of 125 and 250 mg kg^{-1} of body weight per day (Munson et al., 1982). Among the brominated THMs, bromoform is the least potent as a lethal acute oral toxicant (IPCS, 1994).

Principal pathways of human exposure to effluent-borne toxic organic compounds through uptake by plant roots transfer to edible portions of plants and consumption by humans were assessed by Dean and Suess (1985). In addition, nontoxic organic compounds in wastewater can be transformed into potentially toxic chlorinated organic compounds when chlorine is used for disinfection purposes (National Research Council, 1980). The current regulations consider disinfection as an important element of wastewater treatment when necessary to protect public health (EPA, 1992). Byproducts of water and wastewater chlorination are subjects of continuous investigations worldwide (Badaway, 1992; Johnson and Jensen, 1986). Although actual data on the persistence of these by-products on food crops are lacking, their presence should not limit the use of reclaimed water for crop irrigation (Howard, 1989). Since then, most investigators in this field have expressed their concerns. Yet the nature of research conducted focused primarily on the carcinogenic and mutagenic properties of wastewater chlorination by-products as a public health concern. This review attempts to highlight a possible risk which was less considered by past investigators such as the potential effects of escalating trihalomethane levels on crop performance and overall agricultural productivity of selected test crops. Also, information from such studies is likely to provide an understanding of how the presence of these trihalomethane species in treated wastewater may affect physiological plant parameters such as seed germination rates, photosynthesis and plant growth, production of secondary metabolites, vulnerability to oxidative damage, chlorophyll synthesis, bioaccumulation and distribution of THM's in plant tissues.

POSSIBLE EFFECTS OF TRIHALOMETHANE CONCENTRATIONS ON SEED GERMINATION

In order to develop the mechanisms for toxic action for organic pollutants, large-scale experimental toxicity databases are required (Cronin and Dearden, 1995). Wang et al. (2001) validated the use of seed germination rate and root elongation as an indicator to assess phytotoxicity. Numerous investigators have used seed germination rate as a prime parameter in their studies, such as the influence of temperature on seed germination (Godwin et al., 1998; Luiz et al., 1984; Tang and Long, 2008) or the effects of salinity and seed mass on germination (Easton and Kleindorfer, 2009). Shuhe et al. (2009) studied the effects of illumination and seed soaking reagents on seed germination. Closer to home was a research conducted by He et al. (2008) where they studied the effects of cadmium stress on seed germination in rice. Some notable obscure research was also conducted using seed germination as a major para-meter such as the acceleration of germination by applying electric and magnetic fields (Moon and Chung, 2000), effects of radiofrequency and ultrasonic sound enhancement on germination (Kazumitsu and Masahiro, 1988; Tkalec et al., 2009) and even the effects of cigarette smoke on seed germination (Noble, 2001). No literature was found on the effects of trihalomethane species or other common disinfection by-products on seed germination rates. Such a study is now warranted with the use of treated wastewater in agriculture, the possible toxic nature of trihalomethane species on germination requires investigation.

SUSCEPTIBILITY TO BIO-ACCUMULATION AND PHYTOTOXICITY OF TRIHALOMETHANE SPECIES

Evens et al. (2004) investigated the toxicological effects of disinfection using sodium hypochlorite (NaOCl) on aquatic organisms. The aim of their study was to evaluate the toxicity on aquatic organisms of hospital wastewater from services using NaOCl in pre-chlorination. Extensive work has been performed on other classes of persistent organic pollutants in relation to their fate in the environment. A recent study performed by Tokutaka et al. (2008) analyzed the biomagnification profiles of persistent organic pollutants in the aquatic food web of the Mekong delta in Vietnam. Of the persistent organic pollutants analyzed, dichlorodiphenyltrichloroethane (DDTs) was the predominant contaminants followed by polychlorinated biphenyls (PCB's).

Peterson et al. (1995) investigated the physiological toxicity, cell membrane damage and the release of dissolved organic carbon and geosmin by (*Aphanizomenon flosaquae*) after exposure to water treatment chemicals. Peterson and his team focused on the effects of several chemicals used at different stages of the water treatment process on a nitrogen-fixing strain of the cyanobacterium (*Aphanizomenon flos-aquae*). Chemicals they studied included chlorine, potassium permanganate, aluminum sulphate, ferric chloride, calcium hydroxide, hydrogen peroxide and copper sulphate. The amount of literature emerging from the past suggests that the bulk of the studies done concentrated on the toxic nature of mainstream disinfection compounds but not their by-products. Apart from this, there was little evidence in literature to suggest that comprehensive work has been done on the phytotoxic nature of these disinfectant compounds or their by-products. The reasons for this stem from the physical, chemical and transport properties of these by-products in the environment. Scientists have been unable to agree on the potential phytotoxic hazards of these compounds. The health risks to humans of these by-products of disinfection have been clearly defined.

For example, focusing on the predominant trihalomethane specie "chloroform" because of its generally lower humic content, waste water generates less chloroform when treated with chlorine than drinking water sources (USEPA, 1984). With relatively low solubility in water and relatively high vapour pressure, the chloroform generated in water can be expected to partition into the atmosphere (Ballschmiter, 1992) and fluxes of chloroform have, indeed, been observed from potable water (Keating et al., 1997) and sewerage (Haas and Herrmann, 1996). But the concentration of chloroform within soil pores is significantly higher than that in the surrounding air (Frank, 1988; Frank et al., 1989; Frank and Frank, 1990), the enhancement factor ranging from four times to 600 times. The source of this chloroform has been demonstrated to be chlorination of soil acids, mainly humic materials, by hypochlorous acid. This is generated from the chloride ion that is ubiquitous in soil and hydrogen peroxide, in the presence of chloroperoxidase (CPO) enzyme. Chloroperoxidase activity has been observed in many soil extracts (Asplund et al., 1993; Laturnus et al., 1995) and has been shown in the laboratory to catalyse the chlorination by hydrogen peroxide and chloride ion of simple organic compounds, such as acetone, propionic acid and citric acid, to chloroform (Walter and Ballschmiter, 1992).

Similar chlorination of humic acid in the laboratory was demonstrated by Hoekstra et al. (1995), who went on to show by field studies and $Na^{37}Cl$ enrichment of soil, that the production of chloroform is a natural process (Hoekstra et al., 1998). Knowledge of the increased occurrence of chloroform and other trihalomethane species within the soil matrix and the necessity to understand the physiological and phytotoxic effects their presence may have on crop productivity is of paramount importance.

POSSIBLE EFFECTS OF TRIHALOMETHANE CONCENTRATIONS ON PHOTOSYNTHESIS

A new phytotoxicity test was developed by Wundram et al. (1996) which was based on the inhibition of algal photosynthesis. Feasibility was verified by comparative studies with standard phytotoxicity tests with *Lemna* (growth) and *Lepidium* (root elongation), using both solutions with distinct heavy metals and complex mixtures of heavy metals.

Untiedt and Blank (2004) studied the effects of fungicide and insecticides mixtures on apple tree canopy photosynthesis. They accomplished this by applying fungicide/insecticide doses commonly used in commercial orchid practice. Their effects on photosynthesis and dark respiration were evaluated in two seasons with respect to the potential stress they impose on an apple tree. A new technology was employed to continuously examine photosynthesis, dark respiration and carbon balance of apple trees based on six canopy chambers, which enclosed apple trees under natural conditions in the field, with on-line measurements and continuous analysis of CO_2 exchange and automated data acquisition. They discovered that the fungicides mancozeb and flusilazol combined with the insecticide oxydemeton-methyl reduced whole tree canopy CO_2 assimilation mostly at midday and using hourly means, by an averaged 7.4% on the day of its application. This reduction in whole canopy photosynthesis declined with time, restoring most of the original photosynthetic potential within 3-5% in 3 days, hence, indicating acceptable phytotoxicity.

A similar approach may be used to investigate the effects of elevating concentrations of trihalomethane species on photosynthesis for different crops. Carbon dioxide is the raw material required for photosynthesis, reductions in leaf CO_2 exchange by plants under stress reduce growth and production by slowing or stopping photosynthesis (Wittwer and Honma, 1979).

Since plant growth and ecosystem primary productivity are ultimately dependent on photosynthesis, a general relationship between photosynthesis and relative growth rate has been shown empirically and theoretically (Pereira, 1995). Information from such studies could be used to estimate the effects of the presence of trihalomethane species on optimum crop productivity.

Carbonell et al. (1998) studied the effects of arsenic on the wetlands vegetation in Louisiana, by focusing on the availability, phytotoxicity, bioaccumulation and effects on plant growth. They reported that the arsenic chemical form in nutrient solution (MMAA) was the most phytotoxic species to marsh grass, but regardless of the chemical form, an As level in the nutrient solution of 0.2 mg l^{-1} was safe or caused no toxic effects for this marsh grass (it did not reduce plant growth or interfere with plant nutrition). Also, root and shoot As concentrations significantly increased with increasing As application rates (all four species) to the rooting medium.

Bengtson et al. (2005) applied a novel phytotoxicity assay for the detection of herbicides in Hervey Bay and the great sandy straits. The incorporation of the assay into the assessment of surface waters added an important aspect to the study by allowing investigation of the toxicity of cumulative herbicide concentrations andyielding biologically relevant data.

The highest herbicide concentration detected during the study was equivalent to 0.23 µg l^{-1} diuron; a concentration known to inhibit photosynthetic efficiency of the assay biomaterial by approximately 3%. The use of photosynthetic efficiency is a common measure in evaluating the phytotoxic effects of untested organic compounds.

PRODUCTION OF SECONDARY METABOLITES AS A STRESS RESPONSE TO TRIHALOMETHANES SPECIES

Although the production of plant secondary metabolites are generally associated with plant defense responses against herbivores and pathogens, these unique compounds can be involved in a broad array of ecological functions (Bertin et al., 2003). Plant cells produce a vast amount of secondary products. Many of these are highly toxic and are often stored in specific vesicles or in the vacuole. Several studies indicated that this kind of storage functions on one hand as a detoxification of the plant itself in response to the absorption of external toxic compounds and on the other hand a reservoir of, for example, nitrogen-rich molecules (Harborne, 1980).

Good reason exists for the use of some secondary compounds (or, even better, groups of chemically similar compounds) as features of an adaptive mechanism when plants are subjected to stress (Bell and Charlwood, 1980). This review focuses only on the production of phenols, flavonoids and anthrocyanins classes of secondary metabolites as a stress response to elevating concentrations of trihalomethane species.

Riverio et al. (2001) investigated the resistance to cold and heat stress through the accumulation of phenolic compounds in tomato and water melon plants. They reported that thermal stress in both plants induces the accumulation of phenolics in the plant by activating their biosynthesis as well as inhibiting their oxidation. This could be considered an acclimation mechanism of the plant against thermal stress. The concentrations and among compound correlations of individual phenolics in white birch leaves under air pollution stress was studied by Loponen et al. (1998). In order to detect early symptoms of heavy metal pollution in birch (*Betula pubescens*) trees, they studied foliar concentrations of phenolic compounds in polluted and control areas around the Harjavalta copper-nickel smelter, SW Finland. They showed that variation in contents of phenolics was quite large among tree variances of individual phenolics between control and polluted area which differed significantly in the case of seven phenolic compounds. There was no evidence found in literature to suggest an increased biosynthesis of phenolic compounds in plants as a stress response to escalating trihalomethane species concentrations.

OXIDATIVE STRESS AS A RESPONSE TO ELEVATING TRIHALOMETHANE SPECIES CONCENTRATIONS

In every aerobic cell, there is a dynamic equilibrium between reactions generating reactive oxygen species, thus stimulating non-specific oxidations and antioxidants. A change in this equilibrium in favour of oxidative reactions is referred to as oxidative stress (Sies, 1991). Among biochemical systems of increasing interest, the antioxidant responses play a central role in adaptation to extreme environments and in revealing responses of the organisms to stressful conditions. Highly reactive oxygen species are continuously produced in several metabolic pathways of aerobic metabolism, but their potential toxicity to biological components is normally counteracted by the presence of a complex array of antioxidant enzymes and low molecular weight scavengers. Under normal physiological conditions, the efficiency of the antioxidant system reflects the basal prooxidant pressure on the organism which is influenced in different species by several biological and environmental factors including oxygen pressure, regime of light exposure, temperature, food availability, life stage or phase of reproductive cycle (Abele et al., 1998; Dykens and Shick, 1982; Lesser et al., 2001; Regoli et al., 2000; Shick et al., 1996; Vega and Pizarro, 2000; Winston and Di Giulio, 1991). Many forms of environmental disturbance cause the enhancement of intracellular reactive oxygen species generation and an imbalance between prooxidant factors and antioxidant defenses. The consequences of such physiological alterations are peroxidations of lipids, oxidation of proteins, and changes in cellular redox status (Di Guilio, 1991). Collectively, these responses are generally referred to as oxidative stress, which is associated with several pathological states and is frequently observed in response to hypoxia, hyperoxia, UV radiation and exposure to pollutants (Livingstone, 2001). Under optimal growth conditions, the production of reactive oxygen species in cells is estimated at a constant rate of 240 μM s^{-1} O^{2-} and a steady state level of 0.5 μM H_20_2. However, stresses that disrupt the cellular homeostasis of cells result in the enhanced production of ROS up to 720 μM s^{-1} O^{2-} and a steady state level of 5-15 μM H_20_2 (Di Guilio, 1991). The enhanced production of reactive oxygen species (ROS) during stress can pose a threat to cells and many stress conditions enhance the expression of ROS-scavenging enzymes. Because ROS are toxic but also participate in key signaling events, plant cells require different mechanisms to regulate their intracellular ROS concentrations by scavenging of ROS (Storey, 1996). Antioxidants such as ascorbic acid and glutathione, found at very high concentrations in chloroplasts and other cellular compartments (5-20 mM ascorbic acid and 1-5 mM glutathione), are also important for the defense of plants against oxidative stress. Consequently, mutants with suppressed ascorbic acid levels, and transgenic plants with suppressed ROS-scavenging enzymes, are hypersensitive to pathogen attack and abiotic stress conditions. In addition, over-expression of ROS-scavenging enzymes increases the tolerance of plants to abiotic stresses (Hermes-Lima et al., 1998). Several studies have applied this technique in determining stress response to external abiotic factors.

Strycharz and Kalidas (2002) studied the Peroxidase activity and phenolic content in elite clonal lines of *Mentha pulegium* in response to polymeric dye R-478 and *Agrobacterium rhizogenes*. Peroxidases are a ubiquitous group of enzymes known to participate in the oxidative coupling of phenolic compounds during the lignification process. Four elite clonal lines of *Mentha pulegium* were screened for high phenolic content on different concentrations of polymeric dye labeled R-478, a violet colored anthraquinone dye. One of the four clonal lines, MPH-4, showed a significant correlation between phenolic content and peroxidase activity in response to the polydye R-478. A comparison of resveratrol, superoxide dismutase activity, phenolic compounds and free amino acids in *Rehmannia glutinosa* under temperature and water stress was shown by Chung et al. (2006). He noted that superoxide dismutase activity varied from 9.4 to 28.4% under water stress and low temperature. Also, of the 16 individual phenolic compounds myricetin showed the highest concentrations in water deficiency. The antioxidant enzyme activity and phenolic compounds content in red cabbage seedlings exposed to copper stress was examined by Posmyk et al. (2009). They revealed that Cu^{2+} at low doses (0.5 mM) increased the levels of anthocyanin in red cabbage and High Cu^{2+} concentration (2.5 mM) provoked oxidative stress and enhanced thiobarbituric acid reactive substances content in tissues.

In relation to the oxidative stress response of crop plants to increasing concentrations of trihalomethane species in wastewater further studies are required, as there is a lack of evidence in literature to suggest such studies have already been carried out.

EFFECTS OF ELEVATING TRIHALOMETHANE SPECIES ON CHLOROPHYLL SYNTHESIS

A measurement commonly made by plant scientists on crop plants is leaf chlorophyll content. This parameter is fundamental to understanding a plant response to the environment in which it resides (Stevenson, 1982). The light reactions of the plant are carried on by different pigment systems that absorb specific wavelengths of light, that is, blue, green, yellow or red light (Noggle and Fritz, 1983). Chlorophyll absorbs that radiant energy necessary for photosynthesis (Janick, 1979). Loss of chlorophyll in plant leaves decreases the ability of the leaves to absorb light energy which affects photo-synthetic processes and ultimately plant growth (Baker, 1996). Numerous studies have documented the effects of stress on chlorophyll content. Recently, Munzi et al. (2009) examined chlorophyll degradation and inhibition of polyamine biosynthesis in the lichen (*Xanthoria parietina)* under nitrogen stress. The effects of methanol on photo-synthesis of the flag leaves of winter wheat were investigated by Zheng et al. (2008). They showed that photosynthesis was greatly improved by methanol as indicated by higher photosynthetic rates and stomatal conductance. But the enhancement effect of methanol on photosynthesis was maintained for only 3 - 4 days.

Conceicao (2004) looked at the regulation of chloro-phyll biosynthesis and degradation by salt stress in sun-flower leaves. He revealed that sunflowers when exposed to increasing concentrations of sodium chloride showed a reduction of chlorophyll content. It must be noted that no studies were found linking the loss of chlorophyll in leaves to the presence of increasing concentrations of trihalomethane species in water used for irrigation. Hence further study is required in order to evaluate if there is a possible outcome linking the biosynthesis or degradation of chlorophyll to escalating trihalomethane concentrations.

CONCLUSION

Prospective studies should focus on understanding the nature of disinfectants and their by-products commonly found in treated wastewaters or potable water used in crop irrigation. Efforts should be geared towards critically evaluating how the presence of these organic compounds may induce phytotoxic and physiological modifications or transformations in crop plants with the ultimate goal of assessing how overall crop productivity could be affected. Finally, the biomagnification potential of these disinfec-tant by-products in food chain should be clearly understood.

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