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Review

Chemical, biological and physiological indicators of metal pollution in wetlands

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Chemical, biological and physiological responses in terrestrial and aquatic plants may be used as biomarkers to monitor contamination in ecosystems. Some plant parameters such as chlorophyll content, photosynthesis, transpiration, metal uptake and metabolism may be used to determine the level of toxic stress in plants. Similarly, the concentrations of metals in soil may be used to establish the levels of pollution. In this review, we present challenge to researchers involved in environmental assessment to use these simple techniques as a basis for establishing guidelines on environmental pollution.

Key words: Bioaccumulation, biomarkers, environment, metals.

INTRODUCTION

Metals are potentially toxic substances. Excessive concentrations in biological systems are detrimental; destabilize ecosystems because of their bioaccumulation in organisms, and toxic effects on biota and even death in most living organisms (Alloway and Ayres, 1993; Nagel et al., 1996; Banaszak et al., 2000; Kaznina et al., 2005; Liphadzi and Kirkham, 2006; Andra et al., 2010). The bioaccumulation refers to the accumulation of contaminants by species in concentrations that are in orders of magnitude, higher than the surrounding environment (Weigel 2004). This could involve the excessive accumulation of metals in plants. Some of the major sources of metals are irrigation water (when contaminated by sewage and industrial effluent), battery production, metal products, metal smelting, cable coating industries, brick kilns, automobile emissions, re-suspended road dust and diesel generator sets (Duffus, 2002; Sharma and Dube, 2005; Bragato et al., 2006; Madejón et al., 2006). Other sources can include unsafe or excessive application of pesticides, fungicides and fertilizers, and can also include sewage sludge (Murthy et al., 1989; Bart and Hartman, 2003; Silliman and Bertness, 2004; Weis and Weis 2004).

The term biomarker relates to the response within organisms to different level(s) of pollution. It refers to the capability of the organism to indicate the presence and amount of pollutants in the system (Nimis et al., 1993; Sloof et al., 1998). Biomarkers may provide both qualitative and quantitative information on levels of contamination in organisms such as plants (Prasad, 1997; 1999; Maksymiec and Baszynski, 1999a; b).

The term biomarker means any biological response to an environmental chemical below individual level, measured inside an organism or its products (urine, farces, hairs, feathers, etc), indicating a departure from the normal status, that cannot be detected from the intact organism (Van Gestel and Van Brummelen, 1996). Biomonitors or indicators are any species that provides additional information about the health of an environment. In some cases, the assessment of pollutants in soil, sediment and water may not necessarily gives an indication of the state of the environment, without measuring the impact of such pollutant in the tissues of

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living organisms that are part of the food web. The assessment of the bioavailability of the pollutants in living tissues gives a strong indication of their toxicity (Wang et al., 1997; Wang et al., 2009; Mertens et al., 2006). For example, biomonitors in a soil environment are species that reveal the soil quality and other information which is difficult to measure by direct soil analyses (Madejón et al., 2004).

Plant parameters such as chlorophyll content, plant growth and biomass production, photosynthesis, transpiration, metal uptake and metabolism may be used to determine the level of toxic stress in plants (Breckle and Kahle, 1992; Csintalan et al., 1992). A variety of plant species growing in heavily polluted areas may be used as bioindicators to determine the level of pollution. They may also be used in phytoremediation programs. Phytoremediation can be defined as "the efficient use of plants to remove, detoxify or immobilize environmental contaminants in a growth matrix (soil, water or sediments) through the natural biological, chemical or physical activities and processes of the plants (UNEP, 2010). Plants are unique organisms equipped with remarkable metabolic and absorption capabilities, as well as transport systems that can take up nutrients or contaminants selectively from the growth matrix, soil or water. An understanding of such physiological responses of plants (e.g. the content of chlorophyll and photosynthesis processes) and metal bioaccumulation as a result of exposure to metals would be a step towards establishing how these physiological parameters could be used as biomarkers of pollution and serve as an early warning system.

This review outlines the possible physiological responses to metal pollution in wetland plant species with regards to photosynthesis, chlorophyll contents and nutrient concentration in their tissues.

ELEVATED CONCENTRATIONS OF METALS IN SEDIMENT AND SOIL: INDICATION OF POLLUTION

The rapid industrialization and urbanization in some of the developing countries of Africa have resulted in large-scale pollution of the environment and the enrichment of metals in the soil (Adeniyi, 1996; Sanderson et al., 2002; Osuji et al., 2005; Shuping, 2008; Agunbiade and Fawale, 2009). Because environmental sustainability depends largely on a sustainable soil ecosystem, when the soil is polluted, the ecosystem is altered and plant growth is affected (Adriano et al., 1998; Quan et al., 2007). Soil has become a dumping site for industrial and municipal waste, most of which contain metals (Phillips 1999; Yusuf et al., 2003; Okunola et al., 2007).

Roadside soils have been shown to have considerable contamination owing to depositions of vehicles-derived metals (Adeniyi, 1996; Okunola et al., 2007; Agunbiade and Fawale, 2009). Crude oil and their products which are deposited on road surfaces or nearby soils, or in the water bodies have been shown to have elevated levels of metals, consequently becoming toxic to plants and micro-organisms (Odjegba and Sadiq, 2002; Merkl et al., 2005; Osuji et al., 2005; Wang, 2009). Crude oil spills may also add metals to the soil, which become deleterious to soil biota and crop growth (Osuji et al., 2005).

Similarly, many reports from different parts of the world have shown that soils and sediments were heavily polluted with a mixture of metals from industrial effluent and this ultimately affects the physiological parameters of organisms in the soil ecosystem (Table 1). Some of the examples include: cadmium (Cd), nickel (Ni), lead (Pb), arsenic (As) and zinc (Zn) (Reeves and Baker, 2000; Kannavou et al., 2001; Jin et al., 2002; Xendis et al., 2003; An, 2004; Cui et al., 2004).

A study by Chen (2000) in Taiwan showed that Cd, Pb and Zn concentrations in soils contaminated with industrial effluent were at high levels, ranging from 175 - 378, 252 - 3145 and 100 mg/kg, respectively. In this study, more than 800 ha of land were contaminated with industrial effluent waters containing metals such cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb) and zinc (Zn), which were then reflected in brown rice in different concentrations (Chen, 2000).

Extensive and continued monitoring of chemical concentrations of metals in areas with similar industrial activities is therefore, important to alerting people on the presence of dangerous pollutants in the ecosystem. So far, little information is available on the extent of metal pollution in most of the developing world such as those found in Africa.

METAL CONCENTRATIONS IN PLANTS EXPOSED TO ELEVATED LEVELS OF METALS

Plants that grow in polluted environments may show stress symptoms due to bioaccumulation of metals through direct uptake by the plants' roots, stems or shoot (Godbold et al., 1984; Baker, 1987; Dahmani-Muller et al., 2000; Monni et al., 2001) (Table 2). In all plants, some metals are known to perform specific functions within the tissue systems, which may manifest different visual symptoms at elevated concentrations (Table 3). For example, the excessive accumulation of toxic levels of metals in plant tissues, above the established critical levels, may cause growth abnormalities such as leaf senescence, leaf chlorosis (Baker et al., 1994) dwarfism, wilting and death of the whole plant (Godbold et al., 1984; Baker, 1987; Monni et al., 2001; Liphadzi and Kirkham, 2006).

The toxic effect of metals on the physiological functioning within plants is connected to their accumulation in different plant tissues. Studies have shown that plant species will concentrate certain metals in their leaves, stems and roots to varying degrees and,

Table 1. Elevated toxic concentration in sediments and soils exposed to heavy metals.

Metal	Normal concentration (mg/kg)	Status	Reference
	0.6	Sediments	Canadian Council of Ministers of the Environment (1999a)
	0.14 - 2.5	Sediments	Forstner and Wittmann, 1979
Cd	0.07 - 1.10	Soil	Kabata-Pendias and Pendias, 2001
	0.5 - 1	Soil	Liu et al., 1998
	3	Soil	Chen et al,, 1992c
	0.02 - 2	Soil	Alloway, 1995
	34 - 55	Sediments	Forstner and Wittmann, 1979
Ni	1 - 20	Soil	Kabata-Pendias and Pendias, 2001
	43 - 126	Soil	Chen et al., 1994
	60	Soil	Chen et al., 1992c
	1	Soil	Temmerman et al., 1984
	53.3	Sediment	Canadian Council of Ministers of the Environment (1999c)
Pb	10 - 70	Soil	Kabata-Pendias and Pendias, 2001
	24 - 40	Soil	Chen et al., 1994
	120	Soil	Chen et al., 1992c
	50 m	Soil	Temmerman et al., 1984
Co	0.1 - 74	Sediment	Forstner and Wittmann, 1979
	5 - 15	Soil	Temmerman et al., 1984
	37.3	Sediment	Canadian Council of Ministers of the Environment (1999b)
Cr	100	Soil	Chen et al., 1992c
	5 -120	Soil	Kabata-Pendias and Pendias, 2001
Al	4.1 - 5.5	Sediment soil	(Peverill et al., 1999)
Fe		Sediment soil	
Mn	390 - 6700	Sediment	Forstner and Wittmann, 1979
	500 - 800	Soil	Temmerman et al., 1984
	35.7	Sediment	Canadian Council of Ministers of the Environment (1999a)
	16 - 44	Sediment	Forstner and Wittmann, 1979
Cu	2 - 250	Soil	Alloway, 1995
	27.5 - 113	Soil	Chen et al., 1994
	35	Soil	Chen et al., 1992c
	15	Soil	Temmerman et al., 1984
	123	Sediment	Canadian Council of Ministers of the Environment (1999a)
	7 - 124	Sediment	Forstner and Wittmann, 1979
	17 - 125	Soil	Kabata-Pendias and Pendias, 2001
Zn	111 - 214	Soil	Chen et al., 1994
	120	Soil	Chen et al., 1992c
	100	Soil	Temmerman et al., 1984

hence, critical levels may vary among species (Breckle and Kahle, 1992; Baker et al., 1994; Liphadzi and Kirkham, 2006).

Studies have shown that the symptoms that are associated with elevated Mn concentrations (±300 µg/g in plants) include brown spots on mature leaves, interveinal chlorosis and necrosis, deformation of young leaves, growth retardation and leaf tip burning (Foy et al., 1978;

Table 2. Visible symptoms associated with some metals (after Bonnet et al., 2000; Kukkola et al, 2000).

Metal	Toxic symptoms
Cadmium	Chlorosis, necrosis, purple colouration
Lead	Dark green leaves
Nickel	Decrease in leaf area, chlorosis, necrosis, stunting (Nakazawa et al., 2004)
Cr	Alterations in the germination process, stunted growth, reduced yield, mutagenesis (Shanker et al., 2004)
Co	Leaf fall, inhibition of greening, discolored veins, premature leaf closure, and reduced shoot weight
Aluminum	Stunting, yellowing and death of the leaf tips, inhibition of root elongation
Zinc	Stunting, reduction of leaves elongation
Copper	Chlorosis, yellow colouration, inhibition of of root growth, less branched roots
Iron	Dark green foliage. Thickening of roots, brown spots on leaves.
Mn	Marginal chlorosis and necrosis of leaves, crinkled leaves

1988; Wissemeier and Horst, 1992).

Ecotoxicological studies have shown that, plants that are subjected to elevated concentrations of metals in the rhizosphere may have difficulties in the translocation of these metals from the roots to the aerial parts, a situation which may facilitate root damage as a result of excessive metal bioaccumulation (Godbold et al., 1984; Baker, 1987; Baker et al., 1994; Crozier et al., 2002). In such instances, root and plant growth in general will be severely affected by the high concentrations of metals in the roots because roots are also the main routes of metal uptake and nutrients from soil into plants (Crozier et al., 2002).

Based on research findings on some plant species, toxic concentrations of selected metals are: Cd, 0.1; Ni, 10; and Pb, 30, Cu, 20; Fe, 100; Zn; 100 and Mn, 300 µg/g dry weight (Kirkham, 1975; Alloway, 1995; Fageria et al., 2002; Liphadzi and Kirkham, 2005). The bioavailability of these metals to plants depends on factors such as the biological parameters, physico-chemical properties of the metallic elements and their compounds (Duffus, 2002) as well as a range of various factors, such as soil pH, temperature, redox potential, chemical speciation, seasonal changes, sediment type, salinity and organic matter (Otte et al., 1993). At the elevated levels indicated above, certain plants may be well adapted and can employ several strategies in order to survive (Clijsters et al., 1999; Monni et al., 2001).

The demonstration of a dose-response relationship is an essential criterion for establishing whether the metal is responsible for the effects measured. Plant exposure to metal does not necessarily indicate toxicity; it is only after the concentration is above threshold level that toxicity is reached. Visible symptoms are detected only after a threshold is reached. Therefore, the use of plant biomarker is very significant in determining the biological effects of pollutants in the environment at different leaves and stages of growth. Some of the common abnormal-lities and toxic levels in different plant species are shown in Tables 2 and 3. When such metals are present in excessive amounts in the soil then, they have the potential to excessively be taken by plants and later become toxic (Monni et al., 2001).

Plants require essential micronutrients (Zn, Cu, Mn, and Fe) to grow and complete their life cycle (Durham and Snow, 2006). Both essential and non-essential, (Al, Pb, Co, Cr, Cd, Ni) elements are assimilated by plant even though not all are beneficial to plants. Under the deficient supply of an essential metal, an organism shows poor growth and low yield, whereas, an excess supply leads to toxic effect and can sometimes be lethal (Godbold et al., 1984; Baker, 1987; Prasad, 1999; Monni et al., 2001; Davies et al., 2002, Shanker et al., 2004; Liphadzi and Kirkham, 2006).

Several cases of accumulation of heavy metals such as Zn, Cu, Pb, Cd, Ni and Cr, have been thoroughly studied in several wetland plant species, such as *Eichhornia crassipes*, *Typha latifolia, Spartina alterniflora* and *Phragmites australis* (Saltonstall, 2002; Bragato et al., 2006; Jayaweera et al., 2007). The accumulation capacity, strongly depends on the plant's physiological properties (Liu et al., 2005), and inherent soil properties such as pH, organic matter content, texture, temperature, nutrient availability, etc (Sharma et al., 2007). Exceptionally, Cd is particularly dangerous because plants growing in contaminated soils can absorb and accumulate Cd in edible tissues in large quantities without any visible signs, thereby, introducing the metal into the food (Monteiro et al., 2008). It is therefore important to know the amount of metals accumulated in plants at different levels of exposure so as to establish their potential effects in plant growth and development, as well as in phytoremediation programmes.

USE OF BIOMARKERS IN THE DETECTION OF METAL STRESS IN PLANTS

Van Gestel and Van Brummelen (1996) have defined biomarkers as any biological response to an environ-mental chemical at the below individual level, measured inside an organism or in its products (urine, faeces, hair,

Table 3. Elevated toxic concentration in different plant species exposed to heavy metals.

Name of the plants	Plant part	Metal	Toxic concentration (mg/kg)	Reference
Spinach	Shoot	Cr	1.08 - 5.40	Sharma et al., 2005
		Cd	0.01 - 3.42	Qishlaqi et al., 2007
		Pb	0.05 - 1.93	Qishlaqi et al., 2007
		Ni	0.01 - 0.05	Qishlaqi et al., 2007
		Zn	1.95 - 2.73	Qishlaqi et al., 2007
Wheat	Shoot	Cr	0.93	Qishlaqi et al., 2007
		Cd	0.01 - 0.43	
		Pb	0.09 - 25.45	
		Ni	0.53 - 20.33	
		Zn	35.53 - 41.96	
Lettuce	Shoot	Cr	0.17	Qishlaqi et al., 2007
		Cd	0.03 - 2.01	
		Pb	0.03 - 0.65	
		Ni	0.01 - 0.03	
		Zn	2.17 - 3.75	
Celery	Shoot	Cr	0.01- 0.05	Qishlaqi et al., 2007
		Cd	0.01 - 2.19	
		Pb	0.05 - 0.09	
		Ni	0.01 - 0.21	
		Zn	1.11 - 2.97	
Brown rice	Shoot	Cr	0.16	Lin, 1991
		Cd	0.07	
		Pb	0.43	
		Ni	0.54	
		Zn	39.2	
		Co	2.48	

feathers, etc), which indicates a departure from the normal status that cannot be detected from the intact organism. Ernst and Peterson (1994) have defined biomarkers as biochemical, physiological and morphological changes in plants, which measure their exposure to chemicals. The concept in plants may be linked to an "early- warning" signal of pollution induced stress.

Metals can be found in the tissues and fluids of plants as a result of exposure to metals in air, soil and water (Bialonska and Dayan, 2000). At high exposure concentrations, such bioaccumulation of metals could be potentially toxic to plants. In general, enzyme induction in the photosynthetic pathway can be considered as an indirect reaction on toxic metal action (Papi et al., 2002; Tarpley et al., 2005). A set of parameters can be used for rapid screening of metals' toxicity relationship with changes in, for example, plant development, such parameters include, chlorophyll content, mineral concentration, plant growth and photosynthetic activities in plant species (Rijstenbil et al., 1994; Ralph and Burchett, 1998; Ralph, 2000; Foyer et al., 2003).

The presence of elevated levels of metals may interfere with chlorophyll content and adversely affect the photosynthetic activity and plant enzymes, which invariably affect plant provision of nutrients for survival (Papi et al., 2002; Tarpley et al., 2005). Such biological responses in stressed plants may be useful in the monitoring of metal pollution in terrestrial and aquatic ecosystems. Such a biological approach makes it possible to determine whether the natural ecosystem is being altered by pollutants without relying on expensive techniques and conducting long term field experiments (Ernst and Peterson, 1994; Lagadic et al., 1997, 1998; Ferrat et al., 2003; Agunbiade and Fawale, 2009). This could then serve as early warning signs of specific or general stress at each biological level (Ernst and Peterson, 1994).

Understanding the concept of biomarkers and physiological responses of plants is thus critical to the long-term safety and conservation of ecosystems. The biomarkers based on the photosynthetic activity and antioxidative enzyme activities can offer fast and reliable indication of toxicity in polluted wetlands. The content of chlorophyll is an important biomarker of plant exposed to metals. For example, chlorophyll a and chlorophyll b fluorescence were considered to be very sensitive biomarkers when the plant cellular system was exposed to herbicides and heavy metals (Popovic et al., 2003). Metal toxicity has a direct effect on chlorophyll content, and may lead to visible symptoms of chlorosis and necrosis (Table 2), suggesting that a chlorophyll measurement may provide information on the plant physiological changes that are due to exposure to elevated levels of toxic metal concentration. Elevated levels of mineral concentration in different plant organs (above and below ground) may give an indication of metal toxicity effects on plants as a biomarker. At higher toxic level, metals accumulate and damage the cellular components which then reflect as measurable parameter in the plant (Van Assche and Clijsters, 1990; Agunbiade and Fawale, 2009). Scientific evidence has shown that plant species will concentrate certain metals in their leaves, stems and roots to varying degrees and, hence, critical levels are normally used to quantify the toxicity level(s) in the plant organs (Breckle and Kahle, 1992; Baker et al., 1994; Dahmani-Muller, 2000; Liphadzi and Kirkham, 2006).

Several different parameters may be measured to characterize the growth of a plant. The most accurate measure of biomass accumulation is plant dry weight. Other growth parameters include plant height, internode lengths, number of leaves, leaf areas, stem lengths, and stem diameters at prescribed time intervals during the growth period. Ecotoxicigical studies have shown that plants that are subjected to elevated concentrations of metals in the solution may have had difficulties in translocation of these metals from the roots to the aerial parts, a situation which may facilitate tissue damage (Baker, 1987; Baker et al., 1994; Godbold et al., 1984; Crozier et al., 2002) leading to reduction in biomass. In such instances, plant growth will be severely affected.

Measurement of photosynthesis through specialized equipment such as Fluorometer may be a parameter used in biomarker assessment, as the rate of photosynthesis is intimately linked to growth. Inhibition of photosynthesis by metals has been documented (Clijsters and Van Assche, 1985; Prasad and Strzalka, 1999 and Cronk and Fennessey, 2001; Kaznina et al., 2005; Guoa et al., 2007). Additionally, mechanisms involving enzyme induction in the photosynthetic pathway can be considered as an indirect reaction on toxic metal action (Papi et al., 2002; Tarpley et al., 2005). Plants with metal levels above the threshold experience a decrease in photosynthesis rate (A), evapotranspiration (E) intercellular carbon - dioxide concentration (Ci) and stomatal conductance (Qs). Such parameters can be used as photosynthetic biomarkers in comparing plants growing in polluted and non-polluted environment.

CHLOROPHYLL DEGRADATION AS A BIOMARKER OF METAL EXPOSURE IN PLANTS

Chlorophyll is an important component in photosynthesis, which enables plants to convert carbon dioxide and water in the presence of energy from the sun to produce carbohydrates (Hopkin, 1993; Walker et al., 1996). This is used in all plants' essential growth and developmental processes, which gives rise to the plant's distinctive green colour. Any stress that interferes with this metabolic process may produce responses that could be detected by using specialized methods and equipment and such responses may possibly be used as biomarkers of stress (Vangronsveld and Clijsters, 1992). Against this background, it is possible to use chlorophyll concentration in plants as potential biomarker in ecotoxicological studies (Stoltzs and Greger, 2002; Bragato et al., 2006).

The elevated levels of most metals in plants will interfere with chlorophyll content and induce chlorosis (Padmaja et al., 1992; Yan-Hua et al., 2008). The effective method to estimate chlorophyll is through the extraction of the photosynthetic pigments in dimethyl sulfoxide (DMSO) and quantifies the ratio of optical density of chlorophyll a and b at wavelengths of 435 and 415 nm.

This is a reliable method for an estimation of chlorophyll degradation due to pollution (Ronen and Galun, 1984). The advantages of DMSO as a solvent for the extraction of photosynthetic chlorophyll pigments are that the extraction is simple, rapid, and complete, and that the extract is easily stored at low temperatures without degradation under normal laboratory conditions (Hiscox and Istraelstam, 1979; Filbin and Hough, 1984).

Another method used to estimate chlorophyll content in plants includes, the chlorophyll fluorescence technique using the fluorometer. The fluorometer measures the light energy absorbed by chlorophyll molecules in a leaf, which is re-emitted as light-chlorophyll fluorescence (Sánchez-Rodríguez et al., 1999., Hura et al., 2007; Monteiro et al., 2008; Reyes-Diaz et al., 2009; Mielke and Schaffer, 2010). The advantages of this method are that it is simple, non - destructive, non- invasive, rapid and sensitive (Maxwell and Johnson, 2000; Moradi and Ismail, 2007). Plants exposed to higher concentration of heavy metals such as Al, Pb, Zn, and Ni, showed decreasing chlorophyll contents as indicated by the decrease in light fluorescence (Moustakas et al., 1993; Banaszak et al., 2000; Macfarlane and Burchett, 2001). Decreases in pigments due to damage by elevated concentrations of metals suggest that chlorophyll fluorescence measurement can be used as biomarker to monitor chlorophyll contents alterations in polluted ecosystems.

Several cases of decreased chlorophyll content owing to metal toxicity have been reported in the plant kingdom growing in wetland ecosystems (Shalygo et al., 1999; Abdurakmanova et al., 2000; Schoefs, 2001; Cosio et al., 2004; Valavanidis et al., 2005). For example, decreased levels of chlorophyll were observed in *Paspalum distichum*, a wetland plant (Bhattacharya et al., 2009), due to application of contaminated sludge rich in Cr and Mn. In other studies involving rice (*Oryza sativa*), significant reduction in chlorophyll concentrations was recorded at elevated levels of Cd and Cu (Das et al., 1997; Ahsan et al., 2007; Shao et al., 2007).

A study by Stobart et al. (1985) on the effects of Cd on chlorophyll content in the leaves of barley revealed that, this metal inhibited the biosynthesis of chlorophyll, total chlorophyll content and the chlorophyll a/b ratio. Therefore, this parameter offers an opportunity of detection of toxic stress in plants.

ALTERATIONS IN PHOTOSYNTHETIC ACTIVITY AS BIOMARKERS OF EXCESSIVE METAL EXPOSURE IN PLANTS

Photosynthesis is the vital metabolic energy-generation and storing process that takes place in chloroplasts in the presence of sunlight. Metals can affect plant physiological processes such as photosynthesis, which is an important process in plant growth and development (Clijsters and Van Assche 1985; Greppin and Strasser, 1991; Hopkin, 1993; Walker and Hopkin, 2006; Urban et al., 2007). Some visible photosynthesis symptoms associated with metal toxicity are shown in Table 2.

The photosynthesis process in plants exposed to metals is affected through several mechanisms. Photosynthesis is inhibited at several levels, such as: carbon dioxide fixation, stomatal conductance, chlorophyll synthesis, electron transport and enzymes of the calvin cycle (Prasad and Strzalka, 2000; Monnet et al., 2001; Shanker et al., 2004). Singh et al. (2010) reported chlorosis and fragmentation of leaves with mucilaginous discharge in *Najas indica* plants exposed to excessive Pb. In other studies, excessive copper affected the oxidative enzymes in wheat, oat and beans leaves, thus affecting photosynthesis (Shainberg et al., 2001).

The presence of excessive concentrations of metals may interfere with the chlorophyll formation process, thus, causing adverse effects on the photosynthetic activity, which therefore impairs plant growth (Padmaja et al., 1992; Jonak et al., 2004). Duckweed (*Lemna minor*) exhibited chlorosis and reduced growth at higher levels of metal concentrations (Fe, Cu, Cd, Hg, Pb, Ni, Zn and Mn) (Zayed et al., 1998). Similarly, Ibemesim (2010) reported chlorophyll reduction in *Paspalum conjugatum* (Sour Grass) indirectly through metal effects owing to crude oil exposure on the soil. Sun and Wu (1998) reported chlorosis of the leaves of water spinach supplied with higher levels of Ni owing to reduced photosynthesis and a decrease in chlorophyll concentration. Bibi et al. (2010) also reported negative effects of heavy metals (Cd, Cr, and Zn) on the freshwater macrophytic *Nitella graciliformis* J. by decreasing the chlorophyll content and manifesting poor plant growth.

In higher plants, the photosynthetic rates have been reportedly affected at higher levels of metal (Fe, Mn, Cu, Al, Cd, Zn and Pb) concentrations (Belkhodja et al., 1998; Koukal et al., 2003; Sharma and Dubey, 2005; Zhang et al., 2007; Ali et al., 2008). In all plants, metals are known to perform specific functions within the tissue systems and each may manifest different visual symptoms at elevated concentration. The elevated concentrations of metals may affect the photosynthetic apparatus by acting and interfering with the carbon dioxide fixation at several levels (Prasad and Strzałka,1999) resulting in negative effects on the gas exchange site in plant tissue membranes (Baryla et al., 2001). This could ultimately impact the synthesis of starch due to a low translocation rate of photosynthesis in plants growing in polluted environments (Ericson, 1979).

Other studies have shown elevated starch levels in plants grown in Pb, Cd, Zn, and Cu-polluted areas compared to those found in clean sites (Breckle and Kahle, 1991; Smith et al., 1996; Prasad and Strzałka, 1999). One possible mechanism proposed is the interference by these metals in the breakdown of starch to sucrose (Prasad and Strzałka, 1999). Under such circumstances, the net result of impaired photosynthesis is always reduced carbon fixation, which further reduces the plant growth at the whole-plant level. In other studies, which involved a combination of toxic concentrations of metals such as mercury, lead, cadmium and copper, using aquatic plants (*Potamogeton pectinatus* L., *Valisneria spiralis* L., and *Hydrilla verticilata*), it was shown that, all combinations of the metal pollutants caused senescence in all the three species by decreasing chlorophyll, deoxyribonucleic acid (DNA), ribonucleic acid (RNA), protein and dry weight owing to decreased photosynthesis (Jana and Choudhuri, 1982; Juneau et al., 2002).

Studies conducted by Shanker et al. (2004) on the toxicity of Cr to wheat, peas, rice, maize, beans and sunflower plants revealed that the plants' physiological processes such as photosynthesis, growth and development were affected. The affected plants lacked a specific transport system for Cr and hence reduced their photosynthesis activities (Shanker et al., 2004). In this study, Cr toxicity in plants was observed at multiple levels, from reduced yield, through effects on leaf and root growth, to inhibition on enzymatic activities and mutagenesis. Nagel et al. (1996) studied algae *(Chlamydomonas reinhardtii)* and revealed that higher levels of Cr affected photosynthesis through decompose-tion of the absorption spectra in green pigments. Conclusively, Cr affects photosynthesis in terms of CO_2 fixation, electron transport, photo-phosphorylation and enzyme activities (Davies et al., 2002, Shanker et al., 2004).

In a study undertaken to investigate the effect of Cd on growth and photosynthesis of two tomato cultivars at the seedling stage, Dong et al. (2005) listed leaf necrosis, chlorosis, and reddish brown discoloration of the leaf blade as some of the symptoms observed when tomato plants were exposed to a high concentration of Cd. Moya et al. (1993); Prasad et al. (2000); Hattab et al. (2009) also reported that photosynthesis, transpiration, carbohydrate metabolism and other metabolic activities were inhibited by elevated levels of Cd and Pb in tomato plants. Nickel is an essential micronutrient for plants (Eskew et al., 1983), with its toxicity negatively affecting photosynthesis, mineral nutrition, sugar transport and water relation (Pandey and Sharma 2002; Parida et al., 2003; Nakazawa et al., 2004). The influence of Ni on photosynthesis is that, it damages the photosynthetic apparatus on almost every level of its organization, from reduction of leaf chlorophyll concentration and damage of chloroplasts (Madhava and Sresty, 2000; Molas, 2002; Vinterhalter and Vinterhalter, 2005). Similarly, higher levels of Cobalt in plant (Co) alter the structure and number of chloroplasts per unit area of leaf leading to inhibition of the PS2 activity and Hill reaction, which invariably lower the rate of photosynthesis (Plekhanov and Chemeris, 2003). Therefore, photosynthesis is an important physiological plant parameter, and may be used as a biomarker to assess the toxicity of metals in different ecosystems.

CONCLUSION

In conclusion, this review strongly recommends the use of simple biomarkers in assessing aquatic plants exposed to heavy metal. The qualitative and quantitative determination of pollution in wetland ecosystems is dependent on the metal loads present in the system. Their deleterious effects could easily be monitored by scientists and ecotoxicologists using different plant species as biomonitors.

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