CHAPTER 2
LITERATURE REVIEW

Biological monitoring methods

The use of living organisms as bioindicators has become a popular method in assessing the impact of environmental changes on natural communities. These organisms can provide information on the effects of environmental factors on ecosystems. It is difficult to measure this by other methods, *i.e.* physical or chemical analyses (Martin and Coughtrey, 1982). Biomonitoring is particularly important in the detection of toxic stress. Woods and Hoffmann (2000) summarized biomonitoring methods as follows:

(a) Studies on species diversity at different sites, and

(b) Studies on phenotypic variability of single species in different populations

Application of species diversity for biomonitoring

This method is based on a notion of the presence or absence of certain species. These species can be called indicator species. Arndt and Schweizer (1991) noted that there are three kinds of indicator organisms:

1. Pointer organisms are indicators that can provide information on conditions in an ecosystem because they respond to changes in the environment. These can be used in passive monitoring programs.
2. Test organisms are used primarily in toxicological laboratory tests (active monitoring). They take important roles in connection between environmental chemical testing and environmental toxicology.
3. Monitor organisms are used for qualitative and quantitative monitoring of environmental pollutant levels and can be used in both passive and active monitoring.

There has been much research on biomonitoring using vegetation and species diversity. Plants have environmental requirements that are easy to assess, mostly remain in one place, and are more numerous. Bao (1998), who assessed heavy metal stress on

the vegetation structure in Kup Kap Stream valley, Chiang Mai, found that tree density, species richness, and diversity decreased with increases of zinc and lead levels in the soil.

Although lower species diversity can indicate environmental stress, the presence of stress-sensitive species does not always indicate a lack of stress (Woods and Hoffmann, 2000). Plant ability to tolerate pollutants can not be determined based on the presence or absence of species at contaminated sites and particular toxicity tests for one or a combination of pollutants for individual species need to be studied. It is also necessary to study phenotypic variability of species in biomonitoring programs.

Application of phenotypic variability for biomonitoring

This method is based on comparisons of phenotypic variability between populations of a single species from stressed and non-stressed environments. Increased levels of phenotypic variation within a population can indicate environmental stress. Wilsey and Saloniemi (1999) suggested that fluctuating asymmetry (FA) in plant leaves is a potential indicator of environmental stress. Tarun *et al.* (2002) revealed that exposure to lead significantly reduced some plant growth parameters, *viz.* shoot length, number and length of leaves, and biomass, but increased the FA of *Lythrum salicaria* L. (Lythraceae) leaves. Kozlov and Junttila (2002) also suggested that leaf FA is an early indicator of pollution on *Pinus sylvestris* L. (Pinaceae). Although FA has tremendous potential, inconsistent results have been found in some studies and extensive studies may be required to identify traits that will provide consistent indicators of stress (Woods and Hoffmann, 2000).

Plants in environmental research

During the 1970s and early 1980s, numerous plant tests were developed to help safeguard the environment from the chemical threats. Fletcher (1991) pointed out the following five classes of plant-chemical tests and their uses in environmental assessment analyses (Table 1).

Table 1. Classes of plant-chemical tests (Fletcher, 1991)

Class	Purpose	Example Endpoints
1. Biotransformation	Determine influence of plants on chemical fate of environmental pollutants	Change in chemical concentration
2. Food chain uptake	Establish the amounts and concentrations of toxic chemicals which enter foodchains via plant uptake	Chemical concentration
3. Phytotoxicity	Evaluate the toxicity and hazards posed by environmental pollutants to the growth and survival of plants	Death, discoloration, reduced growth
4. Sentinel	Monitor the presence and concentration of toxic chemicals in the environment by toxicity symptoms displayed by plants	Death, discoloration, reduced growth
5. Surrogate	Use inexpensive, socially acceptable plant test as a substitute for an animal or human assays	Chromosome aberrations

Of these five classes, phytotoxicity and sentinel tests have received the most attention and are both in current use by U.S. regulatory agencies: EPA, FDA, and OECD. The required phytotoxicity tests are shown in Table 2.

Table 2. Plant phytotoxicity tests and their descriptions (Fletcher, 1991)

Test	Response measurement
1. Enzyme assay	Enzyme activity
2. Process measurement	Magnitude of a process: photosynthesis, respiration, *etc.*
3. Tissue culture growth	Changes in fresh or dry weight
4. Seed germination	Percent of seeds which germinate
5. Root elongation	Length of root growth during a fixed time period
6. Seedling growth	Changes in height, fresh weight, and dry weight
7. Life cycle	Changes in height, fresh weight, and dry weight, flower and seed numbers

Currently, three of the seven test classes, *viz.* seed germination, root elongation, and seedling growth are being widely used. Phytotoxicity tests can be conducted in both a laboratory (off-site) and field tests (on-site). Gill *et al.* (1991) suggested that *in situ* evaluations of the effects of chemicals from industrial waste sites by using plants is cost

-effective, suitable for multimedia exposure, and ideal for preliminary investigations for hazard identification.

Selection of test plants

Recommended plants for phytotoxicity tests by the U. S. EPA, OECD, and U. S. FDA are listed in Table 3.

Table 3. Test plants recommended by the U. S. EPA, OECD, and U. S. FDA (modified from Fletcher, 1991)

Common name	Scientific name	Family
	EPA	
lettuce	*Lactuca sativa* L.	Compositae
cabbage	*Brassica oleracea* L.	Cruciferae
soy bean	*Glycine max* (L.) Merr.	Leguminoseae, Papilionoideae
kidney bean	*Phaseolus vulgaris* L.	Leguminoseae, Papilionoideae
onion	*Allium cepa* L.	Liliaceae
corn	*Zea mays* L.	Gramineae
oat	*Avena sativa* L.	Gramineae
rye grass	*Lolium perenne* L.	Gramineae
tomato	*Lycopersicon lycopersicum* (L.) Karst.	Solanaceae
carrot	*Daucus carota* L.	Umbelliferae
cucumber	*Cucumis sativus* L.	Cucurbitaceae
	OECD	
lettuce	*Lactuca sativa* L.	Compositae
Chinese cabbage	*Brassica campestris* L.	Cruciferae
cress	*Lepidium sativum* L.	Cruciferae
mustard	*Brassica alba* L.	Cruciferae
radish	*Raphanus sativus* L.	Cruciferae
turnip	*Brassica rapa* L.	Cruciferae
mung bean	*Phaseolus aureus* Roxb.	Leguminoseae, Papilionoideae
fenugreek	*Trifolium foenum-graecum* L.	Leguminoseae, Papilionoideae
red clover	*Trifolium pratense* L.	Leguminoseae, Papilionoideae
vetch	*Vicia sativa* L.	Leguminoseae, Papilionoideae
oat	*Avena sativa* L.	Gramineae
rice	*Oryza sativa* L.	Gramineae
sorghum	*Sorghum bicolor* (L.) Moen.	Gramineae
wheat	*Triticum aestivum* L.	Gramineae
rye grass	*Lolium perenne* L.	Gramineae
	FDA	
lettuce	*Lactuca sativa* L.	Compositae
cabbage	*Brassica oleracea* L.	Cruciferae
cucumber	*Cucumis sativus* L.	Cucurbitaceae
soy bean	*Glycine max* (L.) Merr.	Leguminoseae, Papilionoideae

(continued)

Common name	Scientific name	Family
kidney bean	*Phaseolus vulgaris* L.	Leguminoseae, Papilionoideae
wheat	*Triticum aestivum* L.	Gramineae
corn	*Zea mays* L.	Gramineae
oat	*Avena sativa* L.	Gramineae
tomato	*Lycopersicon lycopersicum* (L.) Karst.	Solanaceae
carrot	*Daucus carota* L.	Umbelliferae
rye grass	*Lolium perenne* L.	Gramineae

Source: (Backer and Bakhuizen van den Brink, 1968)

Table 3 shows that most test plants are common cultivated herbaceous crops. The reasons for using cultivated crops in phytotoxicity tests include:

1. Basic foods for human and animals,
2. Short life cycle and flowing, fruiting, *etc.* can be studied within a short experimental time,
3. Common species in the Western world and certified seeds are readily available from vegetable seed companies,
4. Most of these crops have high seed viability, vigor, and germination rates are higher than with others plants, and
5. The effects on different cultivars and genotypes (clones) of one crop can be studied.

None of the crops listed in Table 3 are native to Thailand. Martin and Coughtrey (1982) noted that the majority of studies in heavy metal tolerance in plants have been restricted to herbaceous rather than woody species. According to Cox (1979), there is a tree genus, *Betula* (Betulaceae), that was found to be tolerant to metals in contaminated or disturbed soils.

Heavy metals

One of the commonest definitions of a heavy metal is a metallic element with a density greater than 5g/cm^3 *e.g.* chromium (Cr; 7.2), zinc (Zn; 7.1), cadmium (Cd; 8.6)

and lead (Pb; 11.4). They can be called trace elements because they constitute only 1% of the total elemental composition (Prasad and Hagemeyer, 1999). There is no doubt that most metals are potentially hazardous to living organisms and not necessarily at high exposure levels. WHO (1973) classified cadmium and lead as members of the group of very toxic metals.

Heavy metal toxicity

Several metals such as manganese, copper, zinc, nickel, molybdenum, and boron are essential for plant growth, but they are toxic when present at high concentration. A large available amount of essential and nonessential metals such as lead, chromium, cadmium, and arsenic can be toxic to plants. Hagemeyer (1999) noted that dose-response-curves of plants and metals (Figure 1) for essential metals, include three phases: deficiency, tolerance, and toxicity. Nonessential metals have only two phases, *viz.* tolerance and toxicity. When doseage is over threshold levels for plants, both kinds of metals are toxic to plants.

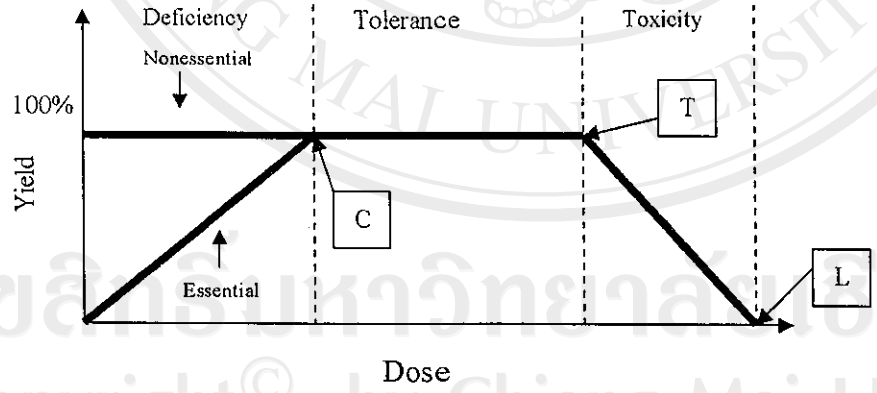

Figure 1. Dose-response pattern for non-essential and essential metals: C= critical deficiency level, T= toxicity threshold, and L= lethal toxicity (Hagemeyer, 1999).

According to Figure 1, between the toxicity threshold level and lethal toxicity level, heavy metals are toxic to plants by depressing photosynthesis, disturbing respiration, and reducing plant growth. Many authors have also revealed that heavy

metal toxicity stress to different plant species and the main toxicity stresses on plants are numerous (Table 4).

Table 4. Heavy metals stress in plants (Prasad and Hagemeyer, 1999)

Main stress	Cause of stress	Reference
Impairment of photosynthesis	Accumulates in leaf, partition in leaf tissue: stomata, mesophyll, and bundle sheath.	Prasad and Strzalka (1999)
Respiration rate changes	Reduce O_2 consumption, CO_2 release, and ATP production	Losch and Kohl (1999)
Growth changes	Reduce or increase growth parameters	Hagemeyer (1999)
Structural and ultrastructural changes	Disfunction of metabolism and physiology	Barcelo and Poschenrieder (1999)
Decrease in transpiration	Reduces water use efficiency; stomatal closure and increase leaf CO_2 concentration.	Poschenrieder and Barcelo (1999)

Although general heavy metal stresses on plants are similar, toxicity levels are not equal between different species. Toxicity degrees of different heavy metals vary with different species.

Heavy metal uptake by plants

Greger (1999) noted there are three categories of plants based on their response to increased heavy metal concentrations in soil, *i.e.*, accumulators, indicators, and excluders (Figure 2).

1. Accumulators: have high concentrations of metals in their tissues at very low external metal concentrations. At high metal concentration, these plants do not increase their uptake, probably due to competition between metal ions at the uptake site.
2. Indicators: have a tissue concentration reflecting the external metal concentration, increasing uptake linearly with increasing metal concentration in the external medium.

3. Excluders: have low uptake of the metal at quite high external metal concentrations. These plants have some kind of barrier to avoid uptake, but when the metal concentration becomes too high, this barrier loses its function, probably due to toxic action by the metal and the uptake increases.

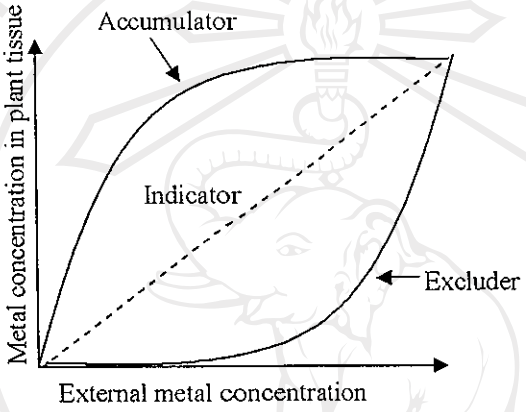

Figure 2. The three different uptake characteristics of plants (Gerger, 1999).

Besides these plant types, Brooks (1998) introduced the term "hyperaccumulators", which are plants that can be grown in heavy-metal contaminated areas and have the ability to accumulate high concentrations of heavy metals (> 1000 mg/kg in dreied leaves) without dramatically retarding their growth and development. These plants have the ability to translocate an element from roots to shoots at high rates. Normally zinc, cadmium, or nickel concentrations in roots are ten or more times higher than shoot concentrations, but in hyperaccumulators, metal concentrations can exceed root levels. Kumar *et al.* (1995) revealed that lead hyperaccumulating plants usually have a higher shoot/root ratio of lead (0.04-0.1) than non-hyperaccumulators. Brooks (1998) summarized the numbers of known plant hyperaccumulators for eight heavy metals and the families in which they are most often found (Table 5).

Table 5. Number of known hyperaccumulator plants (Brooks, 1998)

Heavy metal	Number of species	Families
cadmium	1	Brassicaceae (Cruciferae)
cobalt	26	Scrophulariaceae
copper	24	Cyperaceae, Scrophulariaceae, Poaceae (Gramineae)
manganese	11	Apocynaceae, Cunoniaceae, Proteaceae
nickel	290	Brassicaceae (Cruciferae), Cunoniaceae, Euphorbiaceae, Flacourtiaceae, Violaceae
selenium	19	Fabaceae (Leguminoseae, Papilionoideae)
zinc	16	Brassicaceae (Cruciferae), Violaceae

Kumar *et al.* (1995) noted that most of the commonly known hyperaccumulators belong to the Brassicaceae (Cruciferae). Xiong (1998) found that the lead hyperaccumulator crop species, *Brassica pekinensis* (Lour.) Rupr. (Cruciferae), has 0.09 shoot / root ratio of lead content. This species is a synonym of *Brassica rapa* cv. Chinese cabbage (Kua and Toxopeus, 1994). This species was able to accumulate unusually high amounts of lead in its root and shoot tissues.

Role of hyperaccumulators in phytoremediation programs

Many researchers have detected a few hyperaccumulators with phytoremediation potential for heavy metal contaminated soil and water (Brown *et al.*, 1994; Kumar *et al.*, 1995; Dushenkov *et al.*, 1995; Huang *et al.*, 1997; Blaylock *et al.*, 1997). Phytoremediation is the use of plants to remove soil contaminants and is often referred to as bioremediation and green remediation. Saxena *et al.* (1999) pointed out the advantages of phytoremediation as:

1. large scale application,
2. growing plants is relatively inexpensive,
3. this process is environmentally friendly *i.e.* ecologically safe;
4. plants concentrate the contaminants their tissues, thereby reducing the amount of hazardous pollutants, and
5. concentrated hazardous wastes require smaller reclamation facilities for extracting heavy metals

Brooks and Robinson (1998) reviewed the main problems in phytoremediation technology indicating that there are only few plants that produce high biomass with a high degree of accumulation of the target heavy metals. Some tree species, which have high biomass, are difficult to grow and have a slow growth rate. Because of these limitations, researchers are now searching for hyperaccumulators plants with large a biomass by plant breeding and biotechnology.

Economic benefits of phytoremediation

Phytoremediation's cost must include the entire remedial process from growing, maintainging and harvesting plants to disposing or recycling the metals in the plants. But, this technology is relatively cheaper than other conventional remediation technologies. According to the U. S. EPA, the estimated phytoremediation cost compared with other technologies is shown in Table 6.

Table 6. Estimated cost of phytoremediation (U.S. EPA, 2000)

Contaminated source	Phytoremediation cost (US $)	Estimated cost using other technologies (US $)
10 acres of lead contaminated land	500,000	12 millions

Source: www.clux.in.org/remedi.htm, 2000.

Sources of lead contamination

Lead in the environment occurs naturally and antropogenically. Lead levels in the environment are highly variable. Many researchers have indicated that the main sources of lead contamination are mining and smelting of metalliferous ores, burning of leaded gasoline, disposal of municipal sewage and industrial wastes enriched in lead (Pendias and Pendias, 1984; Seaward and Richardson, 1990). Quynh (1998) reported that the lead contamination in soil ranged from 31.3 to 5,270 mg/kg in an abandoned Pb-Zn mine in Mae Taeng District, Chiang Mai. Pendias and Pendias (1984) recorded that the lead concentration in roadside soil was 7,000 mg/kg in eastern Europe and Wickland

(1990) recorded that lead concentration at 13,380 mg/kg in mining district soil in North America. Huttermann *et al.* (1999) summarized data on lead pollution in eastern Europe (Table 7).

Table 7. Lead contamination in surface soils of eastern Europe (Huttermann *et al.* 1999)

Sources	Concentration (mg/kg d. wt)
Metal processing industries	908-37,300
Urban gardens	17-165
Non-ferricmetal mining	21-3,044

Guidelines for lead contamination in soil

The most well known guidelines for assessing soil contamination are that from the Interdepartmental Committee on the Redevelopment of Contaminated Land (ICRCL) of the UK Department of Environment and that from the Netherlands Housing Ministry. These guidelines are shown in Table 8.

Table 8. Guidelines for lead contamination in soil

Classification as	ICRCL	Netherlands Housing Ministry*	
	Threshold trigger concentration (ppm)	Reference (A) value	Intervention (C) value
Contaminant which poses a hazard to human health	500	85	530

*A= Reference values based on concentration found in natural reserves where the only contamination is from atmospheric deposition.

C= Intervention values where the soils must be cleaned-up

Source: www.contaminated land. co .uk/ std-guid/icrcl-l.htm/ 2000

Lead effects on human health

Lead enters human bodies from eating contaminated foods or drinking water and breathing air or dust. Lead poisoning has been a significant public health problem for centuries since lead is a cumulative poison. Exposure to lead and lead compounds

can be toxic to humans. Some known effects in humans are blood anemia, brain, kidneys, and reproductive systems damages. Childrens are more vulnerable to lead poinsoning than adults. The most sensitive organ in childrens is the central nervous systems (Fishbein, 1978).

Lead effects on plants

Lead contamination in the soil affects plants primarily through their root systems. Xiong (1998) found that roots of *Brassica pekinensis* (Lour.) Rupr. (Cruciferae) were more sensitive to lead than shoots since roots are specialized absorptive organs which are affected earlier and are more sensitive than other organs. Similar results have been observed in *Sesamum indicum* L. (Pedaliaceae) (Kumar *et al.*, 1991), *Sinapis alba* L. (Cruciferae) (Fargasova, 1994), *Lactuca sativa* L. (Compositae), and *Raphanus sativus* L. (Cruciferae) (Nwosu *et al.*, 1995).

Obroucheva *et al.* (1998) studied root growth responses to lead in *Zea mays* L. (Gramineae) young seedlings. They found that lead inhibited primary root growth and affected cell division and elongation. There was no inhibition to lateral root formation, but the branching pattern was more compact and closer to the root tip than in control roots. They also explained that the slow penetration of lead ions into xylem vessels in roots results in low lead content in shoots. Apart from root elongation symptoms, lead can be toxic to plants by decreasing seed germination and biomass, inhibition of chlorophyll synthesis, as well as cell disturbance and chromosome lesions (Pahlsson, 1989; Kumar *et al.*, 1991; Fargasova, 1994; Xiong, 1997).

Because of these toxic effects on plants, sensitive species are locally absent in lead contaminated environments and vegetation structure and biodiversity are gradually changed.

Plant extraction and analysis

Solid sample decomposition is an important step in combined analytical methods. Tuzen (2003) pointed out that in most cases of trace metal analysis, the sample has to be an aqueous solution when using highly sensitive measuring methods, such as

flame atomic absorption spectrometry (FAAS), graphite furnace AAS, Inductive Couple Plasma ICP-AAS, ICP-MS.

Microwave digestion

There are various digestion methods in environmental and biological sample preparation to determine metal concentrations. Tuzen (2003) studied the difference between three biological sample preparation methods: dry ashing, wet ashing, and microwave digestion. He found that the results of these three methods were not statistically significantly different and microwave digestion was preferred. Dry and wet methods are more time consuming and complicated than microwave digestion, which provided safer and cleaner sample preparation, but this method is expensive and needs operational experience.

Atomic absorption spectrometry (AAS)

Atomic absorption spectrometry is still the most widely used technique for the determination of trace meatals. Atomic absorption (AA) spectroscopy uses the absorption of light to measure the concentration of gas-phase atoms. Since samples are usually liquids or solids, the analyte atoms or ions must be vaporized in a flame or graphite furnace. The analyte concentration is determined from the amount of absorption. Applying the Beer-Lambert law: metal concentration is correlated linear relationship with absorbance, directly in AA spectroscopy is difficult due to variations in the atomization efficiency from the sample matrix. Concentration measurements are usually determined from a working curve after calibrating the instrument with standards of known concentrations (Figure 3).

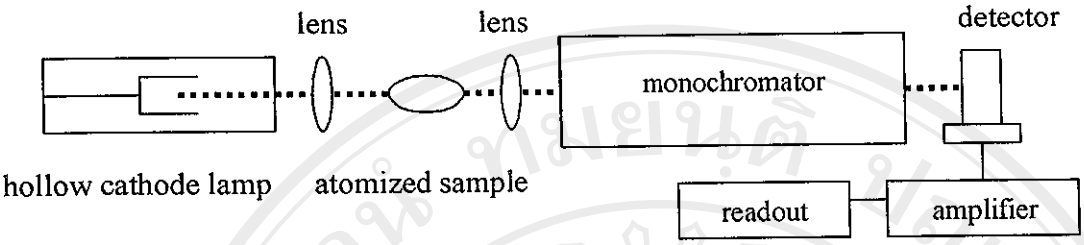

Figure 3. Schematic diagram of flame atomic absorption instrumentation (www.chem.vt.edu/chem-ed/spec/aa.htm/2004).

The general construction of atomic absorption spectrometer is simple. The important components and their function is following:

1. Radiation source-- (hollow cathode lamp or electrodeless discharge lamp) which emits the spectrum of the analyte element.
2. Atomizer-- (flame) in which the atoms of the sample to be analysed are formed.
3. Monochromator -- for spectral dispersion of radiation with an exit slit for selection of the resonance line.
4. Detector-- measurement of the radiation intensity.
5. Amplifier and readout device-- present a reading.