1.1 BASIC UNDERSTANDING OF BIODIVERSITY

The word “Biodiversity” is an abbreviation of “Biological diversity”. A “Biodiversity” is part a of nature which includes the differences in genes among the individuals of a species , the variety and richness of all the plant and animal species at different scales in space, locally in a region, in the country and the world, and various types of ecosystems, both terrestrial and aquatic, within a defined area.

The Earth encompasses an enormous diversity of Angiosperms and Gymnosperms along with the bryophytes, ferns, and fern allies. Estimates of total number of species diverge greatly among authors (Govaerts, 2001; Thorne, 2002; Scotland and Worley, 2003), but a recent estimate has suggested that there are approximately 380,000 species of land plants, comprising ca. 352,000 species of angiosperms, ca. 1,300 species of gymnosperms, and ca. 13,000 species each of bryophytes and ferns/fern allies (Paton *et al*., 2008) . This diversity of living creatures forms a support system which has been used by each civilization for its growth and development. Those that used this “Boundary of Nature” carefully and sustainably survived. Those that overused or misused got disintegrated.

1.2 IMPORTANCE OF AQUATIC BIODIVERSITY

Aquatic ecosystems (habitats and organisms) include our rivers and streams, ponds and lakes, oceans and bays, and swamps and marshes, and their associated animals. These species have evolved and adapted to watery habitats over millions of years. Aquatic habitats provide the food, water, shelter, and space essential for the survival of aquatic animals and plants. The greater the diversity of habitats, whether in water or on land, the greater the biodiversity will be. Coastal estuaries and mangrove swamps, for example, are “edge” ecosystems that link salt- and freshwaters and trap nutrients that allow them to support a rich diversity of aquatic plants and animals. Sustaining aquatic biodiversity is essential to the health of our environment and to the quality of human life. We depend on many aquatic plants and animals, and their ecological functions, for our survival. For example, we use surface waters and their inhabitants to help process our waste products. Each day, aquatic organisms (bacteria and fungi) continuously break down harmful toxins and nutrients that we flush into our sewage systems or discard directly into our rivers and streams.

Aquatic and terrestrial biodiversity are sources of medicine, food, energy, shelter, and the raw materials that we use and need. Although we seldom recognize them, each aquatic species has an important role in making our lives easier, healthier, and more productive. Every living organism has an important role to play, and many are indispensable (Paul *et al.*, 2015).

1.3 THREATS TO AQUATIC BIODIVERSITY

Human activities are causing species to disappear at an alarming rate. Aquatic species are at a higher risk of extinction than mammals and birds. Losses of this magnitude impact the entire ecosystem, depriving valuable resources used to provide food, medicines, and industrial materials to human beings. Runoff from agricultural and urban areas, the invasion of exotic species, and the creation of dams and water diversion have been identified as the greatest challenges to freshwater environments (Allan and Flecker, 1993). Overexploitation of aquatic organisms for various purposes is the greatest threat to marine environments, thus the need for sustainable exploitation has been identified by the Environmental Defense Fund as the key priority in preserving marine biodiversity. Other threats to aquatic biodiversity include urban development and resource-based industries, such as mining and forestry that destroy or reduce natural habitats. In addition, air and water pollution, sedimentation and erosion, and climate change also pose threats to aquatic biodiversity. Following are the same of the important threats to biodiversity.

1.3.1 Overexploitation of species — Overexploitation of species affects the loss of genetic diversity and the loss in the relative species abundance of both individual and /or groups of interacting species. The population size gets reduced because of disturbances in age structure and sex composition. Efficient gears remove quick growing larger individuals. Consequently, the proportion of slow growing ones increases and the average size of individuals in a population decreases. Over-fishing causes change in the genetic structure of fish populations due to loss of some alleles. Thus, genetic diversity gets reduced.

1.3.2 Habitat modification — Physical modification of habitat may lead to species extinction. This is mainly caused due to damming, deforestation, diversion of water for irrigation and conversion of marshy land and small water bodies for other purposes.

Constructions of dams on river impede upstream migration of fishes and displace populations from their normal spawning grounds and separate the population in two smaller groups. Deforestation leads to catchment area degradation due to soil erosion which results into sedimentation and siltation. This not only affect the breeding ground of aquatic organisms but cause gill clogging of small fishes also.

1.3.3 Pollution load — Four forms of pollutants can be distinguished-

1.3.4 Poisonous pollutants — Agrochemicals, metals , acids and phenol cause mortality, if present in a high concentration and affect the reproductive functionality of fish (Kime, 1995).

1.3.5 Suspended solids — it affects the respiratory processes and secration of protective mucus making the fish susceptible to infection of various pathogens.

1.3.6 Sewage and organic pollutants — They cause deoxygenation due to eutrophication causing mortality in fishes.

1.3.7 Thermal pollution — It cause increase in ambient temperature and reduce dissolved oxygen concentration leading to death of some sensitive species.

1.3.8 Metal Pollution — This causes bio-accumulation in organisms that take up these pollutants. Not all animals are affected by these toxic pollutants; they cause growth inhibition, structural damage in plants and also reduce the reproductive ability. Heavy metal toxicity can be seen due to higher levels of mercury. This damages the nervous system of fetuses and children. They also affect the ability to think and learn. At higher levels of toxication they cause permanent damages to brain and even cause death. These factors affect the aquatic biodiversity directly or indirectly. Excessive mortality of organisms due to any of these factors may lead to two type of effects i) extinction of the species / populations ii) reduction of population size.

Hence, there is a significant contamination of fresh water resources as well as artificial eutrophication of lakes and reservoirs and an accelerating accumulation of toxic metals in human food chain. This has assumed serious proportion in the twentieth century and water, one of the most precious and vital components of the biosphere, is being constantly polluted.

Effluents from electroplating plants constitute one of the important sources of heavy metal pollution in surface waters. The increased generation of these effluents containing trace metals poses not only a stress but also a major threat to the biological pool of the ecosystem (Forstner and Wittmann, 1979).

1.3.9 Remediation processes for heavy metals in water:

Physicochemical methods.

Bio remediation methods

1.3.9.1 Physicochemical methods: -

Metals in waste streams do not naturally degrade and are toxic to aquatic life at low concentration for operators of industrial facilities, GE provide effective treatment programs for reducing heavy metal in waste streams to help ensure regulatory compliance. Metals that can be removed from waste water include soluble and/or particulate heavy metal such as Pb, Cu, Cr, Ni, Fe, Mn.

Processes and chemicals to remove heavy metals are:

- Activated carbon or charcoal or coal nitric acid, NaOH and pH buffer Solution
- Acid mine drainage (AMD) by ion for Cu removal line, calcium carbonate, modified silica
- Lime neutralization. Sulfide precipitation to remove mercury arsenic ions from waste water.
- Ion exchange:- Ion exchange is a reversible chemical reaction wherein an ion from water or waste water solution is exchanged for a similarly charged ion attached to an immobile solid particle.
- Reverse osmosis
- Adsorption
- Electric flocculation
- Electro dialysis

Disadvantage of reagent treatment are the larger amounts of reagents required (including corrosive reagents) and the complexity and size of the equipment used to carry out the reaction and remove the precipitate.

1.3.9.2 Bio remediation method:-

The term phytoremediation is a combination of two words – phyto, which means plant, and remediation, which means to remedy. Phytoremediation is the application of plants for in situ or ex situ treatment/removal of contaminated soils, sediments and water. The green plants degrade, assimilate, metabolize, or detoxify inorganic and organic pollutants from the environment or render them harmless. It is a cost effective 'green' technology based on the use of specially selected metal-accumulating plants to remove toxic metals from soils and water. Apart from the advantage of low cost, extraction and concentration of a particular element from the environment, the harvested plants tissue, rich in accumulated contaminants, is easily and safely processed by drying, ashing or composing. The metals can then be reclaimed from the ash, which further reduces the generation of hazardous waste and generate recycling revenue. "The use of specially selected and engineered pollutant-accumulating plants for environmental cleanup is an emerging technology called Phytoremediation" (Elankumaran,2003).

Phytoremediation works best at sites with low to medium amount of pollution, and at sites contaminated with metals. Plants are used either to stabilize or to remove metals from the soil and contaminated water through following five mechanisms:

1.3.9.2.1 Phytoextraction:

Plants are used to remove toxic or heavy metals from soil. It is the uptake of contaminants by plant roots and the translocation/accumulation (phyto extraction) of contaminants into plant shoots and leaves. In this process, plant roots absorb, translocate and store contaminats along with other nutrient and water.

1.3.9.2.2 Rhizofiltration:

Phytofileration/blastofiltration. The use of plant roots to remove toxic or heavy metals from polluted water. It is the process where plants use the roots to absorb, concentrate and precipitate toxic ions from polluted soil and aqueous streams (Dushenkov *et al.*, 1995).

1.3.9.2.3 Phytostabilization:

To eliminate the bioavailability of toxic or heavy metals from soils, using plants. It is the conversion of more toxic form of metal to a less toxic or non-toxic form with in plant tissues(Cunningham *et al.*,1995; Salt *et al*.,1995)

1.3.9.2.4 Phyto-Transformation:

Degradation of contaminants through plant metabolism (applicable to both soil and water). Plants produce enzymes, such as dehalogenase and oxygenase that help catalyze degradation.

1.3.9.2.5 Phytostimulation:

(Plant-assisted biodegradation) also used for both soil and water, which involves the stimulation of microbial biodegradation through the activities in plants rhizosphere.

1.3.9.2.6 Phytovolatilization:

Utilizing plants to extract certain metals from soil and then release them into the atmosphere by volatilization. It is a process where the toxic volatile metals are converted to stable form in the plants and volatilized through the leaves of the plants. eg. Hg^{+2} get converted to Hg^{0} (Meagher and Rugh, 1996).

1.4 CONSERVATION APPROACHES

Aquatic conservation strategies support sustainable development by protecting biological resources in ways that will preserve habitats and ecosystems. In order for biodiversity conservation to be effective, management measures must be broad based.

Aquatic areas that have been damaged or suffered habitat loss or degradation can be restored. Even species populations that have suffered a decline can be targeted for restoration (e.g., Pacific Northwest salmon populations).

An aquatic bio- reserve is a defined space within a water body in which fishing is banned or other restrictions are placed in an effort to protect plants, animals, and habitats, ultimately conserving biodiversity. These bio-reserves can also be used for educational purposes, recreation, and tourism as well as potentially increasing fisheries yields by enhancing the declining fish populations. These bio-reserves are also very similar to marine protected areas, fishery reserves, sanctuaries, and parks. Bioregional management is a total ecosystem strategy, which regulates factors affecting aquatic biodiversity by balancing conservation, economic, and social needs within an area. This consists of both small-scale biosphere reserves and larger reserves (Yadav and Yadav, 2014).

Watershed management is an important approach towards aquatic diversity conservation. Rivers and streams, regardless of their condition, often go unprotected since they often pass through more than one political jurisdiction, making it difficult to enforce conservation and management of resources. However, in recent years, the protection of lakes and small portions of watersheds organized by local watershed groups has helped this situation. Plantation of trees in the catchment area of water body prevent soil erosion and subsequently reduce the problem of siltation in water body resulting in better survival of aquatic organisms. Avoid the establishment of industries, chemical plants and thermal power plants near the water resources as their discharge affect the ecology of water body resulted in loss of biodiversity (Carpenter *et al*., 1998).

The World Resources Institute documents that the designation of a particular species as threatened or endangered has historically been the primary method of protecting the biodiversity. Many specialized programs should be instituted to protect biodiversity. For example, the USDA Forest Service started a cooperative state-federal program with a goal to restore the health of riverine systems and associated species.

Regulatory measures must be taken on wastewater discharge in the water body to conserve biological diversity. Increasing public awareness is one of the most important ways to conserve aquatic biodiversity.

This can be accomplished through educational programs, incentive programs, and volunteer monitoring programs. Various organizations and conferences that research biodiversity and associated conservation strategies help to identify areas of future research, analyze current trends in aquatic biodiversity.

1.5 Ecological grouping of Aquatic plants

The definition of 'aquatic plants' used is plants which are found in the water and in the vicinity of water. They are known as Hydrophytes. Vascular aquatic plants are interpreted as all Pteridophytina (ferns and fern allies) and Spermatophytina (seed bearing plants) whose photosynthetically active parts are permanently or, at least, for several months each year partly or wholly submerged in water or which float on the surface of water.

The definition of wetlands plant is rather more difficult and perhaps potentially dangerous to the future of wetlands; recent legislation in the USA defines a wetland as place where 80 percent or more of the plants growing there are wetland plants. The subject of what a wetland is and what a wetland plant is has been reviewed by Gopal (1990) for India (Cooke, 1996).

Hydrophytic communities are grouped in to the following four forms according to their habitats.

1.5.1 Submerged Rootless Hydrophytes:

Plants of this group are found to growing shallow water. It includes in family *Characeae*, Potamogetonaceae (pond weed), Ceratophyllaceae (hornwort) and Lentibulariaceae (bladderwort)

1.5.2 Submerged Rooted Hydrophytes:

Plants are found in pure stands. Some plants are completely submerged while in some plants vegetative parts are under water and the flowers open above water. It includes families Hydrocharitaceae and Aponogetonaceae.

1.5.3 Submerged and Rooted Hydrophytes with Floating leaves:

They are always found in clear and shallow to deep waters. It includes families Nymphaeaceae and Najaceae, Araceae.

1.5.4 Amphibious/ Wetland Hydrophytes

This group includes plants whose roots and lower part of the stem are always in water. These plants occupy large areas and are very common all along the water lines of all ponds. Alismataceae, Cypraceae, Poaceae, Ornagenaceae, Equisetaceae, Onagraceae, Menispermaceae, Elatinaceae, Eleocarpaceae Lythraceae, Asteraceae, Amaranthaceae, Polygonaceae, Phyllanthaceae and Commelinaceae. The criteria for studying these families are as follows:

1.6 IDENTIFICATION KEYS

The identification keys are biased in favour of easily seen vegetative characters. This has the consequence that many taxa appear several times, the keys are thus lengthened but it is hoped that this will enable plants to be identified in the field without the use of a microscope. Also the user need not worry too much when confronted with difficult to determine alternatives because the plant may appear in both.

Key to major groups

1. Vegetative parts not differentiated into stems with leaves, (leaves, scales or bracts may be found just below flowers)……Key 1
1. Vegetative parts clearly differentiated into stems with leaves or scales 2. Leaves not photosynthetic, reduced to scales (stems green and photosynthetic, bladed or laminate leaves absent)……Key 2
2. Leaves photosynthetic, not scale-like (non-photo- synthetic scales may be present on underground parts or just below the flowers)
3. Leaves jointed between sheath and blade (the base of the leaf is a cylindrical sheath, free or united at the edges, enveloping the stem; above the sheath the blade is free; between the blade and sheath is a joint usually bearing a membranous ligule but sometimes a swelling or row of hairs) ……Key 3
3. Leaves without a joint between sheath and blade
4. Leaf blades peltate (attached to their petioles inside the margin), non-peltate leaves may be present on other parts of the same plant Key 4

4. Leaf blades never peltate

5. Leaves or whorls or clusters of leaves cauline (dispersed along elongated stems at more or less regular intervals)

6. Leaves 3 or more at most nodes ……..Key 5

6. Leaves 1 or 2 at each node

7. Stems creeping, rooting at most nodes (free-floating or bottom-rooted) ……..Key 6 p. 15

7. Stems erect (emergent, floating or sub-merged) usually rooting only at lower nodes

8. Leaves in opposite pairs……Key 7

8. Leaves 1 at each node…….Key 8

5. Leaves or whorls or clusters of leaves not regularlydispersed along elongated stems, most in basal or terminal rosettes or clusters (stems often truncated, corm-like or rhizomatous)

9. At least some foliage leaves clearly differentiated into petiole and blade; blades flattened and laminate……Key 9

9. Leaves without a distinct petiole; blades flattened or not……Key 10

Key 1: Vegetative parts not differentiated into stems with leaves (leaves, scales or bracts may be found just below flowers)

1. At least the basal portion of the plant firmly attached to and flattened against a hard substrate (usually on rocks in flowing water)……..Podostemaceae

1. Plants not attached to and flattened against a hard substrate, free-swimming, free-floating or attached and penetrating a soft substrate

2. Vegetative parts filamentous, repeatedly and regu- ts£"tarly branched; roots absent; with animal catching bladders (mostly submerged)……. Utricularia

2. Vegetative parts not filamentous, frond-like, either | gk5bose or flattened and discoid to elongate; roots absent or present (mostly free-floating, some submerged but then found near the surface)…… Lemnaceae

Key 2: Plants with stems and leaves but the leaves reduced to non-photosynthetic scales, the stems green and photosynthetic (bladed or laminate leaves absent).

1. Scales united below, arranged in regular whorls

2. Lateral branches in regular whorls or absent ……..Equisetum

2. Lateral branches in clusters at nodes …….Cyperaceae

1. Scales not united below, arranged spirally or in pairs

3. Scales in opposite pairs

4. Petals united to a tube below; stamens not exerted; fruit a many-seeded capsule ……..Dopatrium

4. Petals free to base, scale-like; stamens exerted; fruit of 4 mericarps ……..… Myriophyllum

3. Scales spirally arranged, usually at base of stem or solitary

5. Spruit a 1-seeded nut; perianth of hairs, bristles or absent; stem solid …….. Cyperaceae

5. Fruit a many-seeded capsule; perianth of 6, scari- ous scales; stem hollow ……. ……..Juncus

Key 3: Leaves jointed between the sheath and blade (the base of the leaf is a cylindrical sheath enveloping the stem, free or united at the edges; above the sheath the blade is free; between the blade and sheath is a joint usually bearing a membranous ligule but sometimes a swelling or row of hairs)

1. Leaves pinnately nerved, with straight and parallel nerves diverging from the midrib (large emergent herbs)……Canna

1. Leaves not pinnately nerved or if pinnate then nerves not straight and parallel

2. Leaf inserted at the base of the sheath; nerves irregularly branched …….. ……...Polygonum

2. Leaf inserted at or towards the apex of the sheath; nerves indistinct or parallel.

3. Leaves in 3 rows (stems sometimes twisted) ……..Cyperaceae

3. Leaves in 2 rows or spirally arranged

4. Perianth present (all segments petaloid, petaloid and sepaloid or appearing as scales attached to stamens)

5. Perianth entirely petal-like……Pontederiaceae

5. Perianth with both petals and sepals or entirely sepal-like (y Petals showy; anthers stalked, all fertile or some staminodial (without fertile anthers)…… Commelinaceae

6. Petals absent or scale-like; anthers sessile or nearly so, all fertile…….. Potamogeton

4. Perianth absent or reduced to hairs, bristles or scales

7. Fruits sessile in leaf axils or in long-stalked terminal racemes; leaves without silica bodies; stamens 1 or 2 (totally submerged)

8. Fruits in terminal umbels, borne on long flexible stalks; stamens 2……..Ruppia

8. Fruits sessile or nearly so in leaf axils; stamen 1……..…Zannichellia

7. Fruits in spikes, racemes or heads; leaves with silica bodies: stamens 3-6; (flowers in the axils of dry, scarious bracts, grass- or sedge-like plants)

9. Flowers usually each enclosed by 1 bract (glume) variously arranged in spikelets; stems usually solid and triangular in transverse section, without swollen nodes; leaves mostly basal and in 3 rows……Cyperaceae

9. Flowers each enclosed by 2 bracts (lemma outside, palea inside), arranged in spikelets each usually subtended by 1 or 2 empty bracts (glumes); stems usually hollow and round transverse section, with solid and swolle nodes; leaves mostly cauline and in 2 rows ….. …….Poaceae

Key 4: Leaf blades peltate (attached to their petioles inside the margin), plants may also bear non-peltate leaves on other parts of the stem

1. Leaf blades 3-lobed (usually sagittate or hastate) elongate-elliptical

2. Leaves mostly radical (borne near to the root peltate leaf blades borne on rigid petioles and held above the water surface; finely divided submerged leaves absent……Araceae

2. Leaves cauline (borne along the stem); peltate leaf blades borne on flexible petioles, floating; finely divided submerged leaves present …….Cabomba

1. Leaf blades entire, with or without a sinus (the sinus if present not reaching the petiole attachment point) orbicular or nearly so

3. Petioles bearing spines; leaf blades usually more than 10 cm in diameter 4. Leaf blades without spines; plants with milky latex. ……Nelumbo

4. Leaf blades with spines on the under surface plants without milky latex ……. …….Nymphaeaceae

3. Petioles without spines; leaf blades less than 10 cm- in diameter

5. Plants bearing bladder-like, animal catching traps; flowers bilaterally symmetrical; petals united below …… Utricularia
5. Plants not bearing bladders; flowers radially sym-metrical; petals absent or free
6. Flowers single, conspicuous, borne above the leaf blades; most underwater parts covered in mucilaginous jelly……..Brasenia
6. Flowers in umbels, heads or whorls, inconspicuous, borne below the leaf blades; underwater pare without mucilaginous jelly…… ……..Hydrocotyle

Key 5: Leaves whorled (3 or more at most nodes) and cauline (dispersed along elongated stems at regular intervals, not in terminal or basal rosettes or clusters)

1. Whorls with 2 different kinds of leaf (2 floating, entire, green and laminate-the third submerged divided, brown and root-like)…….Salvinia
2. Whorls with all leaves alike or nearly so
2. Plants bearing bladder-like, animal catching traps ……..Utricularia
2. Plants not bearing bladders
3. Leaves terminating in an orbicular lobe hinged along the midrib (catching animals) and 4-6 bristles…….Aldrovanda
3. Leaves not terminating in an orbicular and hinged lobe, terminal bristles absent or less than 4
4. Leaves compound or simple divided into secondary linear or capillary segments
5. Leaves pinnately divided
6. Leaves 2-pinnate or 1-pinnate with at least the lower segments forked; petals tubular towards the base…… Limnophila
6. Leaves 1-pinnate, all segments simple; petals free, caduceus …… Myriophyllum
5. Leaves repeatedly forked or their segments repeatedly forked
7. Ultimate segments with small, marginal thorn-like projections and terminal bristles, all divisions 2-fid (simply forked) ……. Ceratophyllum
7. All segments smooth, lower divisions 5- or 3-fid……. Cabomba
4. Leaves simple and entire
8. Leaves interspersed with hard and sharp spines; seeds inserted on hardened, hook-like outgrowths from the central placenta (retinacula)…….. Hygrophila

8. Leaves not interspersed with spines; seeds inserted directly on the placenta or seeds solitary
9. Leaves lanceolate to ovate (submerged, dark green or reddish, somewhat translucent with 'marginal spines) Hydro charitaceae
9. Leaves linear to capillary
10. Leaves not equally spaced around the nodes (leaves in clusters at each node)
11. Leaves flat, translucent, with toothed margins……Najas

    11. Leaves capillary, not translucent, with entire margins
12. Flowers numerous, subtended by spirally arranged glumes, in ovoid heads …… Cyperaceae
12. Flowers sessile, 1 male and 1 female in leaf axils …….Zannichellia
10. Leaves equally spaced around the nodes (there is a remarkable amount of convergent evolution among aquatics with this particular growth form, identification without flowers is not practical)
13. Flower buds enclosed within a spathe ……. Hydrilla

    13. Flower buds not enclosed within a spathe

    14. Petals united below into a tube
15. Inflorescence very dense, spike-like, mostly terminal; fruit of 4 nutlets …….. Pogostemon
15. Inflorescence lax and racemose, if spike-like then not dense or flowers solitary; fruit a capsule ……. Scrophulariaceae

    14. Petals free or absent
16. Sepals free, not appendaged between lobes; petals and stamens free from the sepals; fruit of 4 mericarps…….. Myriophyllum
16. Sepals tubular, often appendaged between lobes; petals and stamens inserted on the inner surface of the sepal tube; fruit a capsule …… Rotala

Key 6: 'Creepers'; Leaves 1 or 2 at each node (alternate or opposite), stems creeping and rooting at most nodes (plants free-floating or bottom-rooted).

1. Plants free-floating (pleustophytes); floating leaves sessile or nearly so, in 2 rows
2. Floating leaves opposite, 1-lobed, aerial surface with multicellular hairs …….. Salvinia

2. Floating leaves alternate, 2-lobed, the lower lobe in-contact with the water, aerial surface without multicellular hairs……..Azolla
1. Plants bottom-rooted or if floating then floating leaves with petioles and not in 2 rows
3. Leaves elongate, ± terete, without a flattened blade, not differentiated into petiole with blade
4. Leaves 2 at each node, in opposite pairs; plants without bladders ……. Scrophulariaceae
   4. Leaves 1 at each node; plants with bladder-like,animal catching traps …… Utricularia
3. Leaves with flattened blades, usually clearly differentiated into petiole with blade
5. Leaves compound (4 or more leaflets on each petiole)
6. Leaves 1- or 2-pinnate; each leaflet with midrib and lateral veins; stems usually somewhat woody but when in water spongy …… Fabaceae
6. Leaves with 4 terminal leaflets; each leaflet without midrib but with repeatedly forked veins; stems not woody and not spongy … Marsileaceae
5. Leaves simple (sometimes lobed to the midrib)
7. Leaves 2 at each node, in opposite pairs; leaf blades usually sessile or nearly so
   8. Flowers in dense terminal or axillary heads
9. Heads not surrounded by enlarged bracts, usually white and paper-like; ovaries superior ……. Amaranthaceae
9. Heads surrounded by enlarged bracts, not white and paper-like; ovaries inferior…… Asteraceae
8. Flowers solitary in leaf axils or in loose cymes or racemes
10. Petals united to a tube below
11. Flowers radially symmetrical; ovaries inferior ……. Rubiaceae
11. Flowers bilaterally symmetrical; ovaries superior
12. Seeds inserted on hardened, hook-like outgrowths from the central placenta (retinacula); capsules usually elongate…….. Acanthaceae
12. Seeds inserted directly on the central placenta, hard, hook-like outgrowths absent; capsules usually globose …….. Scrophulariaceae
10. Petals free to the base

13. Ovaries inferior; petals usually showy, yellow 'or white……...Ludwigia
13. Ovaries superior; petals either inconspicuous or if showy then pink, red or purple
14. Sepals free to the base; petals free from the sepals……..Elatinaceae
14. Sepals united into a tube surrounding the Svaries; petals borne at the top of the sepal tube between the sepal lobes …….. Lythraceae
7. Leaves 1 at each node, alternate; leaf blades sessile or with petioles
15. Stems rhizomatous, covered with brownish scales; leaves rolled spirally in bud, at maturity bearing sporangia; ferns……...Microsorum
15. Stems or rhizomes without brownish scales; leaves not rolled spirally in bud, not bearing sporangia; flowering plants
16. Stems woody throughout; fruits soft orange to red drupes (rocky river beds)…… Rotula
16. Stems herbaceous or sometimes somewhat woody at the base; fruits hard (nuts, mericarps or capsules), not orange or red
17. Leaves inserted at the base of a tubular sheath which envelops the stem (ochrea)……..Polygonum
17. Leaves not inserted at the base of a sheath
18. Leaves crisped, asymmetrical, with sharp teeth; petals and stamens usually 4 …….. Coldenia
18. Leaves flat, not crisped, symmetrical or nearly so, entire or toothed; petals and stamens either 3 or more
19. Flowers inconspicuous; ovaries inferior; fruit of 2 mericarps……. …….Apiaceae
19. Flowers conspicuous; ovaries superior; fruits capsules
20. Perianth entirely petaloid ……... Pontederiaceae
20. Perianth differentiated into sepaloid sepals and petaloid petals
21. Petals 4, free to base; stamens 6 …… ……..Nasturtium
21. Petals 4-9 united into a tube below: stamens as many as the petal lobes
22. Petals 5, the lobes much shorter than the tube; leaf blades with midrib and pinnate nerves, usually aerial, the margins usually lobed ……. Convolvulaceae
22. Petals 4-9, the lobes much longer than the tube ; leaf blades without a dominant midrib, palmately nerved, usually floating, the margins entire …… Nymphoides

Key 7: Leaves 2 at each node, in opposite pairs regularly dispersed along erect stems (stems emergent, floating or submerged, usually rooting only at the lower nodes)

1. Plants firmly attached to rocks in swiftly flowing water …….. Podostemaceae
1. Plants rooting in a soft substrate or floating
2. Leaves divided into linear segments, the sinuses reaching the midrib or leaf base
3. Leaves palmately divided; sepals 3, free to base; petals 3, free to base; stamens 6 …….. Cabomba
3. Leaves pinnately divided; sepals 4 or 5, free or united at the base; petals 5, free or united at the base; stamens 2 or 4
4. Fruit breaking into 4, 1-seeded mericarps;-petals inconspicuous, free to the base, often caduceus …….. Myriophyllum
4. Fruit breaking into 4, 1-seeded mericarps: petals showy, united into tube below
5. Leaf segments distinctly flattened, the margins notched or toothed …… Acanthaceae
5. Leaf segments filiform, terete or slighth flattened, the margins entire …….. Scrophulariaceae
2. Leaves entire, sometimes lobed but the sinuses not reaching the midrib
6. Leaves capillary, with sheathing stipular base; (totally submerged herbs)
7. Flowers stalked, borne in spike-like inflores¬cences, bisexual…..…… Potamogeton
7. Flowers sessile or subsessile, borne in leaf axils, unisexual……. Zannichellia
6. Leaves not capillary, without sheathing stipular bases (submerged or emergent herbs)
8. Flowers in heads subtended by an involucre of bracts ……..Asteraceae
8. Flowers not in heads or if in heads then not subtended by an involucre of bracts
9. Flowers naked (without sepals and petals or perianth)……Callitriche
9. Flowers with sepals and petals or a perianth
10. Ovaries completely inferior
11. Petals free to the base; petals showy, yellow or white ……Ludwigia
11. Petals united to a tube below; relatively small, whitish ……Rubiaceae
10. Ovaries superior or perigynous
12. Plants with milky latex; capsules swelling and inflated at maturity; seeds with hairs ……. Oxystelma

12. Plants without latex; fruit not a capsule or if a capsule then not swelling at maturity; seeds without hairs
13. Perianth of 4, dry, membranous segments; flowers usually in silver-white heads …… Amaranthaceae
13. Perianth of sepals and petals, never dry and membranous; flowers not in silver-white heads
14. Petals free to the base
15. Sepals free or united only at base …….. Elatinaceae
15. Sepals united into a perigynous tube …….. Lythraceae
14. Petals united and tubular towards the base
16. Fruits breaking into 4, 1-seeded nutlets or mericarps …… Pogostemon
16. Fruits 2-valved capsules
17. Petals radially symmetrical, twisted in bud; petal lobes 4…….. Hoppea
17. Petals usually bilaterally symmetrical, 2-lipped, not twisted in bud; petal lobes 5
18. Seeds inserted on hardened, hooklike outgrowths from the central placenta (retinacula)……..Acanthaceae
18. Seeds inserted directly on the central placenta, hard, hook-like outgrowths absent……..Scrophulariaceae

Key 8 : Leaves solitary at nodes (alternate) regularly dispersed erect stems (stems emergent, floating or submerged, usually rooting only at the lower nodes)

1. Leaves thistle-like (with pinnately arranged marginal spines); flowers in ± spherical heads…….. Sphaeranthus
2. Leaves not thistle-like ; flowers in heads or not
2. Leaf blades of 4, ± equally sized leaflets
3. Each leaflet with midrib and lateral veins; stems usually somewhat woody but when in water spongy; flowers borne in the axils of bracts…….Geissaspis
3. Each leaflet without midrib but with repeatedly forked veins; stems not woody and not spongy; flowers absent, spores borne in sporocarps towards the base of the petioles……. Marsileaceae
2. Leaf blades never of 4, ± equally sized leaflets
4. Leaves compound or leaf blades lobed
5. Leaves palmately divided or palmately lobed

6. Flowers inconspicuous, borne below the leaf blades; ovaries inferior; fruits mericarps …….. Apiaceae
6. Flowers conspicuous, borne above the leaf blades; ovaries superior; fruits heads of nutlets …….. Ranunculus
5. Leaves pinnately divided or pinnately lobed
7. Leaves 2- or more-pinnate with regular, ovate leaflets …. ……..Neptunia
7. Leaves 1-pinnate or pinnately lobed
8. Flowers solitary in leaf axils, rather inconspic-uous; fruits of 4 mericarps …… Myriophyllum
8. Flowers in inflorescences, naked or in the axils of bracts; fruits not of 4 mericarps
9. Flowers in dense heads, each head with an involucre of bracts……Asteraceae
9. Flowers in lax racemes or umbels, without involucres of bracts
10. The leaflets or pinnae entire and very constant in size and shape in any single leaf; stems usually somewhat woody but when in water spongy ……...Fabaceae
10. The leaflets or pinnae lobed and varying in size and shape in any single leaf; stems herbaceous, not spongy
11. Inflorescences racemose; fruits dehiscent capsules…… Nasturtium
11. Inflorescences umbellate; fruits of 2 mericarps ……..…Oenanthe
4. Leaves simple and entire
12. Leaves inserted at the base of a tubular sheath which envelops the stem (ochrea) ……..… Polygonum
12. Leaves not inserted at the base of a sheath
13. Flowers crowded in dense, ± globose heads; heads subtended by an involucre of sterile bracts
14. Peduncles (culms) enclosed in a sheath at the base; flowers unisexual; roots transversely septate ……. Eriocaulaeeae
14. Peduncles not enclosed in a sheath at the base; flowers female or bisexual; roots not septate
15. Leaf blades laterally flattened, with a distinct midrib, without transverse septae; perianth segments not dry and not scale-like …….. Asteraceae
15. Leaf blades vertically flattened (unifacial), without a midrib, with transverse septae; perianth segments dry and scale-like…….. Juncus

13. Flowers not in dense, globose heads; involucre absent
16. Leaves linear, not differentiated into petiole and blade
17. Totally submerged herbs; leaves flaccid, almost transparent
18. Fruit a many seeded capsule …… Hydrocharitaceae
18. Fruit a nut or head of nutlets
19. Perianth of 4, clawed scales, each attached to a stamen …….. Potamogeton
19. Perianth absent 20. Fruits distinctly stalked, in umbels……... Ruppia
20. Fruits sessile or subsessile in leaf axils …….. Zannichellia
17. Emergent herbs; leaves self-supporting, not transparent
21. Leaf blades bearing sensitive, sticky, gland- tipped tentacles, spirally rolled in bud; inflorescences spirally rolled (helicoid) …… Drosera
21. Leaf blades not bearing gland-tipped tentacles, not spirally rolled in bud; inflorescences erect, not rolled
22. Flowers borne in loose cymes; bract margins entire; flowers radially symmetrical…….. ……..Juncus
22. Flowers borne in elongate spikes; bract margins ciliate; flowers bilaterally symmetrical …… Zeuxine
16. Leaves not linear, either differentiated into petiole and blade or blade flattened and laminate
23. Leaf blades bearing sensitive, sticky, gland- tipped tentacles, spirally rolled in bud; inflorescences spirally rolled (helicoid)……. Drosera
23. Leaf blades not bearing gland-tipped tentacles, not spirally rolled in bud; inflorescences erect, not rolled
24. Inflorescence a simple, fleshy spike (spadix) of small and inconspicuous flowers subtended by a thick bract (spathe)……..Araceae
24. Inflorescence without a spadix, spathe absent or if present then of 2, thin segments
25. Perianth not clearly differentiated into sepals and petals, of 4, clawed scales, each attached to a stamen …….. Potamogeton
25. Perianth segments clearly differentiated into sepals and petals, not scale-like, not attached to stamens
26. Floral parts in whorls of 3 or 6 (monocots)

27. Sepals and petals sepaloid; stamens petaloid: leaf blades pinnately nerved with straight, parallel nerves arising from the midrib (large emergent herbs)……. Cannaceae
27. Either sepals and petals petaloid or sepals sepa-loid and petals petaloid; stamens not petal-like; leaf blades with nerves longitudinally parallel from base to tip
28. Perianth of 6 petaloid segments ……. Pontederiaceae
28. Perianth of 3 sepals and 3 petals
29. Ovaries superior; leaves sheathing at the ' base; usually emergent herbs; some stamens usually staminodial …….. Commelinaceae
29. Ovaries inferior; leaves not sheathing; submerged herbs; all stamens fertile or flowers unisexual……Hydrocharitaceae
26. Floral parts in whorls of 4 or 5 (leaf blades with pinnate or reticulate nerves or apparently lacking nerves except for a midrib; dicots)
30. Ovaries inferior
31. Flowers bilaterally symmetrical, adaxially split: anthers united in a ring around the style; petals blue to pink or purple……..… Lobelia
31. Flowers radially symmetrical, not split; anthers -free from each other; petals yellow or white …… Ludwigia
30. Ovaries superior or plants dioecious (female flowers might be lacking)
32. Fruits indehiscent, splitting into 1-seedec nutlets or mericarps
33. Flowers borne in elongate, coiled cymes -"petals united into a tube below; flowers bisexual ……. Boraginaceae
33. Flowers solitary in leaf axils or borne ir. upright racemes; petals free, caducous; flowers unisexual or bisexual (if male then flowers 4-merous) ……Myriophyllum p. 21:
32. Fruits dehiscent capsules or berries, few- to many-seeded
34. Flowers with spurs; leaf bases bearing paired glands; fruits purplish red ……..…… Hydrocera
34. Flowers without spurs; leaf bases withou" glands; fruits not purplish-red
35. Petal tube much longer than the peta lobes; flowers trumpet-like ……Convolvulaceae
35. Petals free or petal tube shorter than tht petal lobes; flowers never trumpet-like
36. Inflorescence a densely packed spike; capsule opening by an apical lid ……. Sphenoclea

36. Inflorescence a panicle of loose axillary cymes or flowers solitary in leaf axils; capsule opening by longitudinal slits or valves …….. Hydrolea

Key 9: Leaves petiolate and not regularly dispersed along the total length of the stem, most leaves in basal or terminal rosettes or clusters; stems often truncate, corm-like or rhizomatous

1. Leaves compound or at least some leaves lobed to the base or to the midrib
2. Leaves irregularly pinnatisect near the sinus; petioles spiny; flowers borne on a spadix surrounded by a spathe…….Araceae
2. Leaves not irregularly pinnatisect; flowers "hot borne on a spadix, not surrounded by a spathe
3. Leaves palmately lobed; fruit of several, free nutlets……...…Ranunculus
3. Leaves pinnately lobed; fruit not of free nutlets or fern without fruit
4. Young leaves not spirally rolled; mature leaves not bearing sporangia; flowers in umbels (leaves ± triangular or irregular in outline; most pinnae pinnately divided or repeatedly forked)……...Oenanthe
4. Young leaves spirally rolled; mature leaves bearing sporangia on the abaxial surface
5. Leaflets very constant in shape from top to bottom of each leaf, margins entire; leaves erect, up to 3 m or more long……Acrostichum
5. Leaflets variable in shape from top to bottom of each leaf, margins usually lobed; leaves submerged, floating or erect, rarely more than 50 cm long……..…Ceratopteris
1. Leaves simple, entire or lobed only at the margin
6. Leaf blades forming a circular mosaic of leaves floating on the surface
7. Petioles not inflated; fruits flattened and winged, without lateral horns; flowers unisexual, without perianth; male flowers with 1 stamen…….…..Callitriche
7. Petioles inflated; fruits with 2 or 4, lateral, hard horns; flowers bisexual, with sepals and petals……...Trapa
6. leaf blades not forming a circular mosaic of whorled leaves floating on the surface
8. Inflorescence a simple, fleshy spike (spadix) of all and inconspicuous flowers subtended by a thick bract (spathe)…….……Araceae
8. Inflorescence without a spadix, spathe absent or r_i : resent then of 2, thin segments
9. Carpels 2 or more, free

10. Leaf nervature almost regularly rectangular; flowers in simple or branched spikes……...Aponogetonaceae
10. Leaf nervature not regularly rectangular; flowers not in spikes
11. Leaf margins lobed or notched but not sagittate; flowers not 3-merous (petals and sepals 5, stamensnumerous)……..Ranunculus
11. Leaf margins entire or sagittate; flowers 3-merous
12. Fruits 1-seeded nutlets; flowers borne in whorls……..Alismataceae
12. Ovaries containing many seeds, the ovules scattered over the inner surface of the carpels; flowers borne in simple umbels.....……Limnocharitaceae
9. Carpels 1 or more, united
13. Styles united into a disk with radiating stigmas; stamens more than 6 ……..Nymphaeaceae
13. Styles not united into a disk, without radiating stiginas; stamens 6 or less
l4. Ovaries inferior; male flowers if present 3-merous……..…Hydrocharitaceae
14. Ovaries superior; male flowers if present 5-merous
15. Perianth differentiated into sepals and petals; bisexual flowers 4-8-merous……Nymphoides
15. Perianth either sepaloid or petaloid; flowers 3-merous
16. Perianth sepaloid or bract-like, not showy; leaves stiff and distinctly ribbed; petioles not inflated (Sri Lanka)……..…..Hanguanap.
16. Perianth petaloid, showy; leaves not stiff and not distinctly ribbed; petioles often inflated (widespread)…….….Pontederiaceae

Key 10: Leaves without distinct petioles and not regularly dispersed along the total length of the stem, most leaves in basal or terminal rosettes or clusters; stems usually truncate, corm-like or rhizomatous

1. Leaves flattened throughout most of their length (parallel-sided in transverse section, sometimes 'V'-shaped or curved, not terete, not semicircular, not triangular, not rectangular)
2. Leaves widest towards the apex (spathulate, obovate or obtriangular)
3. Leaf blades bearing sensitive, sticky, gland-tipped tentacles, spirally rolled in bud; inflorescences spirally rolled (helicoid)……..roserap.

3. Leaf blades not bearing gland-tipped tentacles, not spirally rolled in bud; inflorescences erect, not rolled
4. Leaves densely pubescent; plants free-floating rosettes……...…Pistia
4. Leaves glabrous; plants attached to the bottom
5. Fruits splitting into 2 or 4 mericarps
6. Mericarps 4, flattened and winged; flowers unisexual; perianth absent ……..Callitriche
6. Mericarps 2, ± hemispherical; flowers bisexual; perianth of 5 sepals and 5 petals…….……Phyla
5. Fruits capsules; petals united into a tube below
7. Plants with bladder-like, animal catching traps; petals with abaxial spurs; leaves alternate……..….Utricularia
7. Plants without animal catching traps; petals without spurs; leaves opposite……..……Scrophulariaceae
2. Leaves widest at or towards the base or leaves parallel-sided
8. Midrib running asymmetrically towards one side of the leaf; inflorescence cylindrical apparently arising near the base of the leaf……...Acorus

8. Midrib running symmetrically up the middle of the leaf or midrib absent 9. Leaves with distinct spiny margins

10. Stems emergent, elongate, hard, woody; ovaries superior……..Pandanus
10. Stems submerged, corm-like or if elongate then soft, flexible, never woody; ovaries inferior……....Hydrocharitaceae
9. Leaves with entire or minutely toothed margins
11. Flowers in compact, spherical to discoid heads
12. Heads sessile surrounded by and embedded in foliage leaves (minute herbs)……..Trithuria
12. Heads borne on stalks or culms, held above the foliage leaves
13. Flowers unisexual; petals small, not showy, white or black; seeds solitary ……Eriocaulonp.
13. Flowers bisexual; petals showy, yellow; seeds 2 or more in each loculus …….Xyris
11. Flowers not in compact, spherical to discoid heads

14. Flowers densely packed in simple cylindrical, superposed spikes on a single axis; flowers unisexual……Typha
14. Flowers not densely packed, not in cylindrical inflorescences
15. Perianth united into tube below
16. Perianth blue to purple; stamens 3; small annuals without a rootstock ……..Burmannia
16. Perianth white tinged with red; stamens 6; rootstock a bulb……Crinum
15. Perianth or free sepals and free petals
17. Perianth of scarious, bract-like segments; leave usually with transverse septae ……..Juncus
17. Perianth of sepaloid sepals and petaloid petals (petals sometimes reduced or absent); leave-without septae
18. Carpels free; fruit a head of 1-seeded nutlets…….Alismataceae p. 37 18. Carpels united; fruit a many-seeded capsule
19. Ovary superior; stems usually emergen with self-supporting leaves …….Commelinaceae
19. Ovary inferior; stems submerged with sub-merged or floating leaves …….Hydrocharitaceae
19. Leaves not flattened throughout most of the: length (circular, semicircular, ellipsoidal, triangular or rectangular in transverse section)
20. Leaves capillary (hair-like and flaccid)
21. Flowers tightly packed in globose heads; head subtended by an involucre of bracts
22. Perianth dry, papery, inconspicuous, greyish or blackish; flowers unisexual; roots septate……..…….Eriocaulon
22. Perianth petaloid, showy, yellow, blue or rarely white; flowers bisexual; roots not septate……Xyris
21. Flowers not tightly packed in globose heads : if so then the heads not subtended by an involucrt of bracts
23. Flowers radially symmetrical, bisexual; periard of 6 ± equally sized dry and scale-like segments……..Juncus

23. Flowers not radially symmetrical, unisexual : bisexual, each subtended by 1 glume-like bract; perianth absent or of scales or bristles……..……Cyperaceae

20. Leaves not capillary (awl-like or elongate, self-supporting)

24. Leaves with 4, longitudinal, septate gas canals (visible in transverse section); leaf bases swolle and usually bearing spores……..…Isoetes

24. Leaves without 4, longitudinal, gas canals; leaf bases not swollen, never bearing spores

25. Perianth absent or of scales or bristles

26. Leaves in 2 rows, equitant; inflorescence superposed spikes (male above and female below)……..Typha

26. Leaves in 3 rows or arranged spirally not equitant; inflorescence not of superposed spikes

27. Perianth absent or of scales or bristles……....Cyperaceae

27. Perianth of 6 scarious segments……. Juncus

28. Flowers radially symmetrical; stamens 6; rootstock a bulb……..Crinum

25. Perianth sepaloid and/or petaloid, not of scales or bristles

28. Flowers bilaterally symmetrical; stamen 1; root-stock a corm……Philydrum

1.7 DNA BARCODING

The cost of DNA purification and sequencing has dropped considerably in recent years so that identification of individual species by DNA barcoding has become an independent, subtler method than solely morphological based classification to distinguish closely related species, which also defines the systematic relationships by analysis of genetic distance. The key element for a robust barcode is a suitable threshold between inter- and intra specific genetic distances. Sequence variation between species has to be high enough to tell them apart while the distances within species must be low enough for them to cluster together (Meyer *et al*., 2005). The mitochondrial coxidase subunit I (COI) gene has proven to be a reliable, cost effective, and easily recovered barcode marker to successfully identify animal species, but its application in the plant kingdom is impeded by a slow nucleotide substitution rate, which is insufficient for the diagnosis of individual species (Kress *et al*., 2005 and Chase *et al*., 2005).

However, the Consortium for the Barcode of Life (CBOL) plant-working group recently proposed seven leading candidate sequences for use as barcoding markers (Hollingsworth, 2009). Four plastid coding genes (rpoB, rpoC1, rbcL and matK) and three non coding spacers (atpF-atpH, psbK-psbI and trnH-psbA) have been selected based on previous investigations among different plant families (Lahaye *et al*, 2008). However, the utility of each of these sequences for individual families of species within the plant kingdom is hardly predictable (Pennisi, 2007).

Although there have been attempts to use the single locus of mat K, a combination of two loci, rbcL and trnH-psbA, and even multi-loci combinations (Fazekas *et al*, 2008) as barcoding sequences, the use of a unified barcode for the identification of all the land plants would be difficult due to conflicting needs of different researchers. For example, an optimal barcode marker that has been determined empirically to distinguish plants at the family level may prove less useful for making accurate species level identifications. Most of the proposed plant barcode markers were designed primarily for identifying distantly related organisms in biodiversity hotspots such as Panama (Kress *et al*, 2009). So far, little attention and only a few studies have been devoted to developing unifiedbarcodes suitable for making identifications within a family, within a genus, or between closely related sister species.

Moreover, a test of seven other candidate barcoding sequences in the family of Myristicaceae was applied to eight species within a genus and yielded two suitable barcodes (Newmaster, 2008). Recently, it has been shown that all three markers (rbcL, trnHpsbA and matK) can discriminate 4 sister species of Acacia across three continents (Steven *et al*, 2009). The marker matK has been reported to distinguish 5 Dendrobium species (Asahina *et al*, 2010). Accurate taxonomy and sample identification are crucial to analyses the ecology and evolution of species, higher-level taxonomic groups and communities. Morphological taxonomy has long been used to define species using consistent differences in external characters, but the scale of the challenge of identifying and classifying all species in this way is prohibitive (Tautz *et al*., 2003). While approximately 1.5 million, predominantly insect, 90 species have been described to date, these represent only a small proportion of estimated global diversity and molecular studies continue to reveal cryptic taxa inseparable on the basis of morphological taxonomy (Bergmann and Russell, 2007; Starrett and Hedin, 2007), increasing the magnitude of the challenge.

Approaches based on genetic markers, particularly DNA sequence data, are increasingly used to augment or replace morphological taxonomic analyses (Tautz *et al*., 2003). As discussed by (Vogler and Monaghan, 2006), at least 3 conceptually different but related approaches have been used. DNA taxonomy uses patterns of variation in DNA sequence data to define taxa *a priori*, without reference to morphological data; though it can 100 enable the identification of diagnostic morphological characters.

This approach uses no predefined level of difference (e.g. % sequence divergence) to define taxa, but attempts to identify independently evolving lineages. Because the topology of an individual gene tree can differ significantly from population and species trees, identification of such lineages is best approached using data for multiple loci (Meyer and Paulay, 2005; Hickerson *et al*. 2006).

A second approach captures the sequence diversity present in a group of samples by identifying molecular operational taxonomic units (Floyd *et al.*, 2002), defined as a group of sequences differing from one another by a specified maximum number of base pairs (Blaxter, 2004). DNA barcoding is the third approach and rather than defining taxa *a priori*, it uses sequence similarity at a single 'barcode' locus (in Metazoa, usually the mitochondrial locus 115 cytochrome *c* oxidase subunit I, *coxI*) to allocate unknown specimens to morphologically determined voucher taxa (Floyd *et al*., 2002; Blaxter, 2003; Hebert *et al*., 2003).

CBOL selected three criteria for finding a proposed DNA barcode for land plants: *Universality*, i.e. which loci can be easily sequenced from land plants; *Sequence quality and coverage*, i.e. which loci will produce bidirectional sequences with no or few ambiguous base calls; and *Discrimination*, i.e. which loci will allow most species to be distinguished from one another (CBOL Plant Working Group 2009).From a pool of various laboratories, they retrieved sequence data of 907 samples representing 445 angiosperms, 38 gymnosperms, and 67 cryptogam species. Using these samples, they evaluated 7 possible sequence candidates: four are parts of coding genes (*matK*, *rbcL*, *rpoB*, and *rpoC1)* and the other three are the noncoding spacer regions (*atpF-atpH*, *trnh-psbA*, and *psbK*-psbI). From these 7 loci, two short coding regions of cpDNA (*matK* and *rbcL*) have been selected as proposed DNA barcodes by the CBOL Plant Working Group (2009).

On average, 72% of plant species can be distinguished using a two-locus barcode. By using the same two locus barcode for known species in the same geographic region, a greater degree of discrimination is possible approaching 100% in some cases (Chase and Fay, 2009).

1.7.1 Chloroplast

The well conserved chloroplast genome is the starting point for many research studies involving plants. Chloroplasts are photosynthetic organelles that provide essential energy for plants and algae and are believed to have originated through endosymbiosis between a 6 photosynthetic bacterium and a non-photosynthetic host.

Chloroplasts, also known as plastids, contain hundreds of different proteins that carry out a variety of metabolic functions involving starch metabolism, biosynthesis of amino acids, chlorophyll, fatty acids, etc., in addition to their primary involvement in photosynthesis (Palmer, 1987). A single cell in leaves contains anywhere from 400 -1,600 chloroplasts. The angiosperm chloroplast genome varies little in size, structure, and gene content, ranging from 135 to 160 kilobases (kb). Chloroplast genomes contain a large 20 - 30 kb inverted repeat, which divides the remainder of the genome into two regions, one large single copy (LSC) and one small single copy (SSC) region. Most of the genes within the chloroplast genome code for photosynthetic proteins, while the remainder are transfer RNA or ribosomal RNA genes. In the 1980s, an explosion of data resulting from two parallel and complementary developments occurred: (1) the intensive sequencing and characterization of 10 chloroplast genomes (six angiosperms, a bryophyte, and three algae), culminated in the complete sequencing of the three land-plant genomes, and (2) the physical and partial gene mapping of more than 1,000 different chloroplast genomes addressing issues of genome and plant evolution (Palmer, 1991). As of 2013, there are approximately 115 species of plants whose chloroplast genome sequences have been completed. Among this group, *Nicotiana tabacum, Lactuca sativa, Oryza sativa, Spinacia oleracea,* and *Arabidopsis thaliana* have served as model organisms in plant molecular biology. In 1988, Jeffrey Palmer and his colleagues, while studying chloroplast genomes of different plants, found that swelling or shrinkage of the chloroplast genome was occurring primarily in the non-coding inverted repeat regions. They also found a correlation between the size of the genome and the size of variation in inverted repeats (Palmer, 1988).

Smaller genomes 7 had fewer numbers of repeats whereas the larger genomes such as Geranium had inverted repeats as large as 76 kb. Changes in the genome complexity of a chloroplast can occur by mutations involving the addition of new sequences and deletion of existing ones, rather than by the gradual drifting of repeated elements that eventually become a single large copy.

A vast majority of the length polymorphisms are short, ranging from 1 to 10 base pair sequences in non-coding regions. Large scale changes in inverted repeats is less-frequent and are detectable through restriction fragment length polymorphisms (RFLPs) and involves the additions or deletions of 50 to 1200 base pairs (Fazekas, 2008). The chloroplast genome is generally smaller than that of mitochondrial or nuclear genomes. Land plant cpDNA evolve more slowly and rarely undergoes internal rearrangement when compared to mitochondrial and nuclear DNA.

This is contrary to its relative abundance in relation to size of the mitochondrial and nuclear DNA (Palmer, 1991). The conserved genome of cpDNA combined with its smaller size suggests that the chloroplast genome may be evolving under strong constraints to prevent or eliminate any unnecessary changes in sequences. Cullis (2005) suggests that one possibility of this phenomenon is that the plants with smaller chloroplast genomes efficiently reject additional DNA by either not incorporating or by rapidly removing DNA sequences which then migrates to the nucleus as part of an overall strategy to maintain a small genome. In some cases cpDNA can migrate to the nucleus with the help of cpDNA encoded proteins. This size constraint is further exemplified by non-photosynthetic flowering plants whose plastid genomes have eliminated most or all of their photosynthetic genes (Palmer, 1991). Chloroplast DNA is a large component of total cellular DNA and has a conservative rate of nucleotide substitution. These slow rates of molecular evolution are ideal for plant studies at or beyond the family level. The characteristics discussed above make the chloroplast genome a viable platform for phylogenetic related studies. Chloroplast genomes are not the only genome from which we can derive genetic information for analysis. Nuclear and mitochondrial genomes can also be used. Although the latter genomes are not desirable because of higher rates of mutations, internal rearrangement, and larger genome sizes.

1.7.2 Chloroplast v/s Mitochondrial and Nuclear Genomes

Mitochondrial and nuclear genomes are larger than chloroplast genomes, which can potentially increase inaccuracy and cost of sequencing. Mitochondrial DNA (mtDNA) also evolves very rapidly in comparison to cpDNA. In 1988, Palmer and colleagues reported that mitochondrial and chloroplast genomes contain two sets of vital genes.

The first sets of genes encode many proteins crucial to the fundamental bioenergetic processes of the cell, and the second set encodes the components necessary for gene expression. Given the importance of these genes, Palmer entertained the idea that these highly valuable processes would change very slowly over the course of evolution, unlike animal mtDNA which evolves very rapidly. Although sequence comparisons of several plant mitochondrial genes suggest a much lower rate of point mutations in plant mitochondria than animal mitochondria, point mutations in plant mitochondria are still higher when compared with point mutations rates in the chloroplast genome.

Thus, the mitochondrial genome is less reliable for intra-specific studies in plants. 9 Nuclear genomes are closely intertwined with the chloroplast DNA. That is the nucleus controls the synthesis of many proteins found in the chloroplast (Cullis, 2009). In a sexually reproducing diploid, one allele at each locus in the nuclear genome comes from the mother and the other allele comes from the father, which is known as biparental inheritance. But not all DNA is inherited equally from both parents. Mitochondria and chloroplast DNA are inherited from one parent and are found outside the cell nucleus. Biparental inheritance can be dismal for barcode analysis as the genetic make-up is inherited from two sources compared to chloroplast genomes which come from a single source. Nuclear genomes also are more difficult to work with because they have high rates of nucleotide substitutions and deletions as well as high rates of gene rearrangement (Zhang, 1996). The completed genome sequences of *Arabidopsis thaliana* and *Oryza sativa* have helped researchers search for evolutionary evidence of the transfer of genes from the original endosymbiont to the nucleus. The proteins encoded by the *Arabidopsis* nuclear genome that are closely related to proteins encoded by the chloroplast genomes (44 plastid genes) of other species as identified by the Arabidopsis Genome Initiative provides evidence for genetic transfer between the chloroplast and the nuclear genomes (Cullis, 2009).

The genes missing from the *Arabidopsis* chloroplast genome but present in the nucleus are presumed to represent organelleto-nuclear gene transfers which occurred sometime after the divergence of the organellecontaining lineages (Cullis, 2009).

The use of DNA based identification of species has increased in the past decade. DNA barcoding is one of the emergent methods for identification of unknown species and to distinguish between various clones within the same species (CBOL Plant Working Group, 2009). Although the animal kingdom has a standard DNA barcode in place, there is no such standard for land plants.

1.7.3 Maturase K

The first of the two DNA barcoding regions proposed by the CBOL Plant Working Group is the Maturase K (matK) gene of the chloroplast genome. The matK gene, formerly known as *trf* K is approximately 1500 base pairs long and is located within the intron of the chloroplast gene trnK on the large single-copy section adjacent to the inverted repeat (Hilu and Liang, 1997).

A homology search of the putative ORF's gene product showed that the amino acid sequence at the carboxyl end is similar to portions of maturase-like polypeptides and might be involved in splicing group II introns (Neuhaus and Link, 1987). Open reading frames of 509 codons of matK were first characterized in tobacco, *Nicotiana tabacum* L., (Sugita *et al.*, 1985). A slightly longer reading frame of 524 codons was later found in mustard, *Sinapis alba* L. (Neuhaus and Link, 1987). The presence of matK in the parasitic Epifagus, a taxon that has lost approximately 65% of its chloroplast genes, speaks for the functional significance of the matK gene (Hilu and Liang, 1997). The gene matK has also been effective in addressing many systematic questions in various species. For DNA Barcoding, the matK gene has evolved at a higher rate than most of the other genes within cpDNA currently used in plant systematic studies. Olmstead and Palmer (1994) reported that out of 20 genes used in molecular systematics, matK had the highest nucleotide substitution rate. Johnson and Soltis (1992) reported that the matK gene evolves approximately three fold faster than the rbcL gene. Comparing eleven complete sequences representing multiple 11families and nine partial sequences representing monocot families from GenBank, Hilu and Liang (1997) showed that matK had a relatively high rate of substitution in the conserved regions of the gene.

They ultimately concluded that the relatively conserved 3' region and the less conserved 5' region provided two sets of characteristics that can be used to discriminate between and within species. In terms of amplification, a sequencing success of 85-88% was found for the matK gene region through the use of up to 10 combinations of primers or with more sophisticated chemistry at the amplification stage (Piredda, 2010).

Maturase K is the most rapidly evolving plastid coding region and regularly shows high levels of discrimination (CBOL Plant Working Group, 2009). In contrast to the reports by others who found that the matK gene region was difficult to amplify (Wicke, 2009), the CBOL Plant Working Group reported that 90% of the angiosperms tested in 2009 were successfully distinguished from one another using a single primer pair to amplify and sequence the matK gene (CBOL Plant Working Group, 2009). The transition-transversion ratio for matK gene sequences was calculated to be 1.25, which is lower than the expected value of 2.0 for relatively recently diverged sequences and exceeds the value by 0.4 for highly substitution-saturated sequences (Homquist, 1983). The higher rate of substitution, reasonable size, and higher level of discrimination underscore the usefulness of the matK gene for DNA barcoding purposes.

1.7.4 Ribulose-1,5-bisphosphate carboxylase/oxygenase

The 1400 base pair Ribulose-1,5-bisphosphate carboxylase/oxygenase gene, also known as RubisCO or rbcL (L=large subunit), is responsible for fixation of carbon dioxide in plants. The enzyme RubisCO consists of eight identical chloroplast-encoded large subunits and eight small 12 subunits (Clegg 1993). RubisCO is the most abundant protein found on earth and can comprise up to 50% of the total soluble protein found in leaf tissue (Tabita, 2007). This gene is responsible for the first steps in photosynthesis in plants and algae and has been well conserved throughout evolution. Some chloroplast-encoded genes are interrupted by introns but this is not the case with RubisCO, which illustrates one of several important advantages of the rbcL gene (Clegg, 1993). Another advantage of using the rbcL gene as a DNA barcode is its ability to be used as a two locus barcode with the gene matK. Using matK as one of the dual barcodes can allow for better intra-specific species studies because of its higher rate of variability, while rbcL will allow for higher discriminatory power between species because of its conserved nature.

Direct estimates of nucleotide substitution rates for rbcL confirmed this (Zurawski, 1984). Because of the conserved rate of nucleotide substitutions, universal primers which should be applicable to nearly all flowering plants can be constructed for use in the polymerase chain reaction (PCR). Hasebe and colleagues (1992) obtained rbcL sequences from the chloroplast genome of Pteridophytes, vascular plants that have the greatest loss of phylogenetic information because of their long evolutionary history. Two phylogenetic trees were developed by the neighbor-joining method and the parsimony method using rbcL gene sequences from 58 species representing almost all families of the Leptosporangiate ferns. These two methods produced almost identical phylogenetic trees, which gave three new insights into the evolutionary trends of Leptosporangiates: 1) two morphologically distinct heterosporous water ferns, Marsilea and Malvinia , are sister genera; 2) the tree ferns (Cyatheaceae, Dicksoniaceae, and Metaxyaceae) are monophyletic; and 3) polyploids are distantly related to the Gleichenioids in spite of the similarity in morphologies. This shows that the high rate of conservation in rbcL is important 13 within photosynthetic plants. In addition, sequence variation in the rbcL gene is easily amplified by PCR. The CBOL Plant Working Group (2009) found a 90-98% success in PCR and DNA sequencing using a single pair of primers in angiosperms. The rbcL gene is capable of providing high levels of discriminatory power, which increases the potential of DNA barcoding.

Throughout the chloroplast genomes, the rbcL gene is well characterized. With the improvements in designing universal primers, discriminatory ability of the rbcL gene sequence, and bidirectional sequencing for the elimination of ambiguous base calls (CBOL Plant Working Group, 2009), rbcL is a valued choice in DNA barcoding schematics. Although not the most variable region, it is a frequent component of the best performing multi-locus combinations for species discrimination (CBOL Plant Working Group, 2009).

1.7.5 Application and Global Benefits of DNA Barcoding

Utilizing barcodes for routine species identifications is the most widely accepted of the potential applications.

Suggestions have also been made to use DNA barcodes for species descriptions, phylogenetic analysis and conservation efforts although these applications are highly controversial (Rubinoff, 2006). DNA barcoding can help to achieve many of the Millennium Development Goals (MDGs) and reach the objectives of the Convention on Biological Diversity.

1.7.5.1 Controlling Agricultural Pests: Lessening Poverty and Hunger (MDG 1) Pest damage to agriculture costs farmers the equivalent of billions of US dollars each year. DNA barcoding can identify pests in any life stage, making it easier to control them.

The global Tephritid Barcoding Initiative will contribute to the management of fruitflies and will provide border inspectors with tools to identify and stop fruitflies at the border. Ensuring pest-free trade will gaurantee better access to global markets.

1.7.5.2 Identifying Disease Vectors: Combating Diseases (MDG 6) many of the most serious human and animal infectious diseases such as malaria are transmitted through vector species. DNA barcoding enables non-taxonomists to identify these vectors, thereby helping to understand and curb disease-carrying pests and pathogens. A global Mosquito Barcoding Initiative is building a reference barcode library that can assist public health officials to control this important disease vector more effectively and with less reliance on insecticides.

1.7.5.3 Protecting Endangered Species: Hunting for bushmeat has reduced primate populations by 90% in some parts of Africa. Law enforcement can use DNA barcoding to distinguish bushmeat in local markets obtained from endangered species, thereby contributing to the conservation of biological diversity. A DNA barcoding initiative for conservation has recently been launched, with the goal of creating a barcode library for critically endangered species.

1.7.5.4 Monitoring Water Quality: Drinking water is rapidly becoming a precious resource. The health of lakes, rivers and streams is often measured by studying the organisms living in them. DNA barcoding is being used to document these indicator species that can otherwise be difficult to identify. Environmental agencies can use barcoding to improve their water quality assessments, and to create and enforce better policies that ensure sustainable supply of safe drinking water.

1.7.5.5 Sustaining Natural Resources :

Over-harvesting of natural resources such as fish and hardwood trees is leading to species depletion, extinction and the economic collapse of industries that rely on them. Natural resource managers and regulators can monitor the illegal trade of processed products using barcoding.

Reference barcode libraries are being constructed for fish (FISH-BOL) and hardwood trees (TreeBOL), with the goal of improving the management and conservation of these natural resources.

1.8 HEAVY METAL POLLUTION IN AQUATIC HABITATS

The term “heavy metals” has received widespread usage for metals that are potentially toxic in high doses. The elements having a specific gravity greater than 4.5 g/cm^3 (sometimes defined as 5.0 or 6.0) are called heavy metals (Streit and Stumm, 1993). Except for their specific gravity, heavy metals have no common chemical property or behaviour in biological or ecological systems.

Therefore some authors prefer to classify the metallic group of elements into “A (oxygen-seeking), B (nitrogen/sulphur-seeking) and borderline metals”, according to their ability to become a part of a chemical complex (Nieboer and Richardson, 1980). Many authors use the term “trace metals” as a synonym for heavy metals.

However, this term should perhaps be restricted to its original meaning, i.e. for metals required by plants in extremely small amounts, “trace” amounts. In this use the term “heavy metals” when referring to copper (Cu), nickel (Ni), iron (Fe), manganese (Mn), zinc (Zn), cadmium (Cd) and lead (Pb). Aluminium (Al), which has a specific gravity of 2.7 g/cm^3, is a metal. Heavy metals such as Mn, Zn, Cu, Fe and Ni are essential micronutrients for plants, but are toxic at higher concentrations and disturb most of the primary physiological processes of plants (Marschner, 1995). Cd is non-essential element for plants, and may be toxic or lethal even when absorbed in small amounts.

Industrial wastewaters which have heavy metals are an important source of environmental pollution. These industrialization and urbanization have led to an increase in metal contamination of aquatic environments. Pb, Cd, Cu, Hg, Cr, Ni, and Zn are the main trace elements that are the most harmful to public health. (Doyurum *et al*., 2006). The increased amount of heavy metals has resulted in toxicity of soil, air and water (Corradi *et al*., 1995).

Unlike organic pollutants, which in most cases can eventually be destroyed, metallic species released into the environment tend to persist indefinitely. They circulate and eventually accumulate throughout the food chain, thus posing a series of threats to animals and man. During recent years, the intensive industrial activities, such as electroplating, microelectronics, battery manufacture, dyestuff, chemical, metallurgical, pharmaceutical (Cojocaru *et al.*, 2008), metal plating, metallurgical alloying, ceramics, photography and other, greatly contribute to the increase of heavy metals in the environment.

The available fraction of heavy metals is that which can be readily mobilized in the soil environment and taken up by plant roots. Heavy metal mobility is a very important factor relevant to the chemistry in these metals in the soil, because it is related to the risk of movement of these metals down the soil profile and the subsequent contamination of the ground water deposits. The availability of metals to crop plants is also related to the accumulation of metals in the human food chain to potentially hazardous levels.

Pollution of environment by toxic metals arises as a result of various industrial activities and has turned these metal ions into major health issue (Weisberg *et al.*, 2003). Although several adverse effects of the toxic metals have been known for a long time, exposure to heavy metals continues, and is even increasing in some parts of the world, in particular in less developed countries.

Heavy metal pollution is also a multi-element problem in many areas. These elements easily taken up by plants and then enter the food chain, resulting in a serious health issue for humans. The increasing levels of heavy metals in the environment, their entry into the food chain, and the overall health effects are of major concern to researchers in the field of environmental biology.

Their extensive uses in industry have created pollution problems, which are sometimes associated with plant toxicity and risks concerning animal and human health. In recent years, research on these elements has received a great deal of interest as they are considered to be potentially highly toxic elements. This may be ambiguous though, because all elements are harmful when their concentrations in living organisms exceed certain thresholds.

After the Industrial Revolution, elevated quantities of heavy metals have been emitted into the atmosphere, changing the natural balance and accelerating, some times dramatically, their biogeochemical cycling. Moreover, it is important to recognize that heavy metal pollution usually includes several different elements (multi-element pollution) especially in areas of metalliferous mining.

1.9 OCCURRENCE OF METALS IN THE ENVIRONMENT

There are approximately 90 elements found in the earth' s crust; of these only 9 account for over 99 % by weight, while the rest account only for 0.14 % and these are called 'trace elements'. Some of these trace elements are considered to be essential for the normal healthy growth of organisms while others are non-essential. Essential elements are those that have been found to have some essential biochemical function in the bodies of organisms.

The elements that are universally accepted as essential to all organisms are: C, H, O, Ca, Cu, Cl, F, Fe, Mg, S, Zn, Mn, Mo, N, P, K and Na. Of these C, H, O, Ca, K, P, Mg, S, Cl and N are macro-elements, while the others are essential trace elements or micronutrients (Jones, 1982). Apart from those, there are elements which are essential only to certain organisms. These are: Co (to bacteria and animals), Cr (to animals), Mo (to plants), Ni (to plants), Se (to animals), B (to plants) and I (to animals) (Alloway, 1996). If essential elements are not supplied to the organisms, they can develop deficiency symptoms and possibly die. As the supply of the elements increases organisms increase their production and biochemical functions are restored.

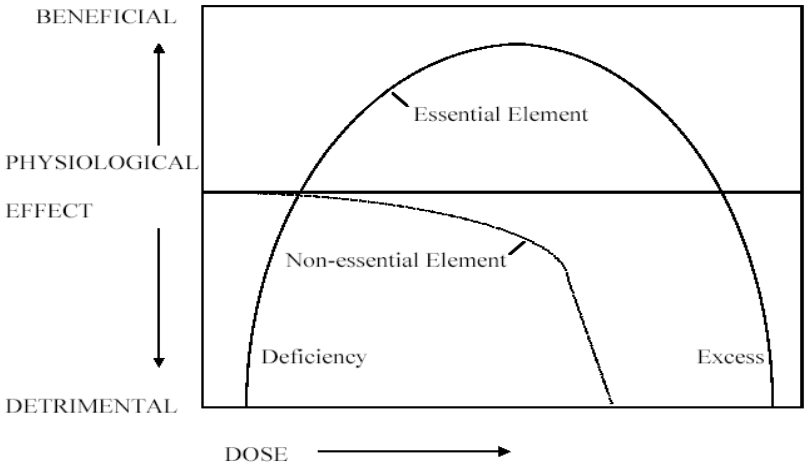

Figure 1: ***The function of essential and non-essential elements in organisms (*****From Sadler et al., 1985*****).***

There is a range of concentration for each of these elements, which is optimum for each organism to grow, after which toxicity symptoms start to develop. For non-essential elements, on the other hand, there is a range of concentration that the organism tolerates, after which toxicity symptoms develop. These typical dose-response functions are shown in Figure 1. Heavy metals are trace elements with a specific density of greater than 6 g cm^{-3} (Sadler *et al*., 1985) suggested that under this definition all metals in the periodic table, especially those in Groups I and II should be considered as heavy metals.

	Cd	**Ni**	**Zn**
Magmatic	<0.3 a	5-15	52
Sedimentary	<0.3 a, 0.1 e	7.9 e	20-30 e
Soil	0.06-1.1 a	0.2-450 a	17-125 a
Soil Solution	0.2-6 a	3-25 a	4-270 a
Ocean (µg kg^{-1})	0.1		2 d, 11 b
Earth' s Crust	0.2 b, c, 0.1 e	75 c, 80 e	70 b, c, 75 e

Table 1: ***Occurrence of Cd, Ni and Zn in the environment (µg g^{-1}).Sadler*** **et al.,** ***1985***

Three heavy metals were chosen to be studied: Cd, Ni, and Zn. Cadmium and Zinc are very imperative and mobile elements which have been widely studied, Ni (like Zn) except from being mobile in the soil is essential for plant growth is considered as a very serious contaminant.

1.9.1 Cadmium

Cadmium belongs to Group II of the Periodic Table. It is found mainly in magmatic and sedimentary rocks with concentrations up to 0.3 μg g^{-1}. In the weathering process of the rock minerals it moves readily into the soil solution, where it is normally found in the form Cd^{2+}. This chemical form is the most common form of Cd. Other ionic forms that may be found in the soil solution are: $CdCl^{+}$, $CdOH^{+}$, $CdHCO_3^{+}$, $CdCl_3$, $CdCl_4^{-}$, Cd $(OH)_3$ and $Cd(OH)_4$ (Kabata-Pendias and Pendias, 1992). Cadmium has had a wide range of uses in industry, including paints and pigments, electroplating, plastic stabilizers and silver-cadmium batteries.

Cadmium is a by-product of Zn refining in the Zn mining industry. This process follows the formula:

$$ZnS \xrightarrow{O_2} SO_2 + ZnO \xrightarrow[heat]{C} CO_2 + Zn/Cd \xrightarrow[heat]{distill} Zn + Cd$$ (O'Neil, 1993).

In recent years there has been an elevated input of Cd into the environment through increased use of phosphatic fertilisers, wastes (including sewage sludges) and aerial emissions.

1.9.2 Nickel

Nickel is an essential element for plant growth, and it belongs to Group VIIIa of the Periodic Table. Its occurrence in the earth's crust ranges from 1400-2000 μg g^{-1} in the ultramafic rocks to 5-15 μg g^{-1} in rocks with higher silicate contents (acidity), like the granites. The ion Ni^{2+}, which is the result of weathering of the rock minerals, is relatively stable in the soil solution. Organic matter has a great influence on this element as Ni can be strongly adsorbed by it (Leeper, 1978). As a result, Ni distribution in the soil profile is related to the organic matter content, and also to oxides and clay minerals. The commonest ionic forms of Ni (except for Ni^{2+}) are: $NiOH^{+}$, $HNiO^{2-}$, $Ni(OH)^{3-}$ (Kabata-Pendias and Pendias, 1992).

Nickel may be a serious airborne contaminant of industries using Ni-carbonyl compounds. Nickel-carbonyl has been reported to be carcinogenic, although this is doubted (Neil, 1993). Nickel is widely used in industry, as it is a metal which does not corrode as much as Fe. It is, therefore, used in the production of alloys, on which it confers them stain and corrosion protection.

1.9.3 Zinc

Zinc is an essential element for the plant growth. It occurs naturally in ore minerals especially sphalerite (ZnS). Weathering produces Zn^{2+} which can substitute for Mg^{2+} in silicate minerals in the soil. The hydroxide $Zn(OH)^{+}$ is also adsorbed on Fe and Mn oxides and clay minerals containing Al and Si lattices (Lindsay, 1979). Other ionic forms in the soil solution are: $Zn(OH)_2$ and $ZnHPO_4$. Zinc is adsorbed strongly onto organic matter and clay particles in the soil and this adsorption is related to the Cation Exchange Capacity of the system in acidic media and is influenced by organic ligands in alkaline media.

Industrial uses of Zn include corrosion protecting coating and manufacture of brass and other alloys. Zinc has a very similar environmental chemical behaviour to Cd, as both elements normally occur together. Zinc plays an important role in many biochemical functions within plants. Zinc is an essential component over 300 enzymes.

1.10 TOXIC EFFECTS OF HEAVY METALS

1.10.1 Humans

The higher concentration of chemicals in environment, the more children with inconsistent physiological reaction were found. Children living in polluted areas more often had hypertrophy of tonsils, increased lymphatic nodes and liver size and dismorphic features. Children dwelling in environmentally unfavorable areas showed significant decrease in immunity. Negative influence of toxicants took the form of secondary immune-deficiency state, which was expressed by repeated respiratory infections, etc. Due to the accumulation of heavy metals in humans, effective rehabilitation treatment, which includes pectin-vitamin tablets, natural adaptogenes and antioxidants, have been tested and proposed.

1.10.2 Plants

Uptake and excess of metals by plants initiate a variety of metabolic reactions, finally leading to global phytotoxic responses e.g. dwarf growth and chlorosis. The reduction in the photosynthetic pigments due to disorganization of chloroplast was reported by many researchers: which suggested that chlorophylls ("a", "b" and total chlorophyll) get inhibited due to metal induction. The chloroplast is the organelle most affected by metal contamination. Heavy metals bind to the thiol (–SH) and nitrogen containing groups in the enzymes in the plants thus blocking the catalytically active sites. This way they are reported to be "**enzyme blockers**". Some essential metal ions form metallo-enzymes after their incorporation into the cells and cause inactivation of enzymes. Many of them can form the lipid soluble organ metallic compounds that accumulate within the cells and organelles impairing their function.

Heavy metals severely inhibit root growth (Bennet, 1987 and Punz, 1993). Bio avaibility of heavy metal in soil, uptake of heavy metal at phytotoxic level, growth retardation, affects on palisade and spongy parenchyma cells in leaves (Ahmed, 2003 and Ladygein, 2004), collated deposition in the vascular bundles and change in vacuoles with electron dense material along the walls of xylem and phloem vessel (Ladygein and Semenova, 2003).

Cd tolerance mechanisms may differ depending on the species because of Cd banding with certain plants products, the amount of Cd retained in active site can be small, then Cd toxicity is alleviated (Cobbett, 2000). Cadmium decreases the chlorophyll content (Astofi *et al*., 2005). Cadmium drastically changed the lipid composition of membranes; the content of palmitic acid increased and the contents of linoleic and linolenic acids decreased in all classes of lipids. Cadmium showed break down of cortex in the light micrograph of root of barley plant (Shridhar, 2007).

In the case for Ni (Robertson, 1985; Robertson and Meakin, 1980) have demonstrated that Ni inhibits root growth in part by inhibiting cellular division in the root apex, yet they were unable to determine whether the effect was primary or secondary. Furthermore, several studies have indicated that Ni inhibits photosynthesis (Bishnoi, 1993).

However, toxic amounts of Ni have been shown to increase the amount of carbohydrates in the leaves (Moya, 1993), which may appear contradictory to the previous studies. In most of these enzymes, zinc makes up an integral component of the enzyme structure. The most distinct zinc deficiency symptoms stunted growth and little leaf are presumably related to disturbances in the metabolism of auxins and indole acetic acid (IAA). Zinc is a major industrial pollutant of the terrestrial as well as aquatic environment. General symptoms of zinc toxicity are wilting; necrosis of old leaves, and reduced plant growth. Zinc toxicity inhibits chlorophyll formation in young leaves (Kaya, 2000) Photosynthesis and transpiration is also reduced by high concentrations of zinc.

1.10.2.1 Terrestrial Plants:

The presence of excessive amount of Cd in soil causes many toxic symptoms in plants, such as reduction of growth, especially root growth, disturbances in mineral nutrition and carbohydrate metabolism (Moya, 1993) and may therefore strongly reduce biomass production. The reduction of biomass by Cd toxicity could be the direct consequence of the inhibition of chlorophyll synthesis (Padmaja *et al*., 1990) and photosynthesis (Baszynski, *et al*., 1980). Reduction in photosynthetic rate for different plant species under exposure to Cd and Zn stress (Sawhney, 1990 and Sheoran, 1990).

In contrast, (Haag-Kerwer *et al*., 1999) reported that photosynthesis in *Brassica juncea* was not affected even when it was exposed to 25 μmol/L Cd, while transpiration showed significant decline, in particular. Metal stress in Lactuca sativa and in Lupinus albus increases the level of aspargine in root exudates (Toppi and Gabbrielli, 1999) and lubimin and 3-OH- lubimin in Datura stramonium, (Mithofer *et al*., 2004).

1.10.2.2 Aquatic Plants:

Vascular aquatic macrophytes may accumulate considerable amounts of heavy metals in their tissues (Kovacks *et al*., 1984). In the recent past, several of the submerged, emergent and free floating aquatic macrophytes are reported to bioconcentrate heavy metals in natural waters as well as after exposure to wastewaters (Greger, 1999).

Some aquatic or semi aquatic plants such as *Eichornia crassipes* (Dierberg *et al*., 1987), *Nasturtium officinale* and *Mentha aquatica* (Saygıdeger, 2005) can take up heavy metals from contaminated solutions.

Metal accumulation in the form of Ca- oxalate crystals found on the aquatic plant *Echhornia Crassipes* (Mazen and Maghraby, 1997). It has been found that highest bioaccumulation is seen with zinc, copper, iron and nickel, which are found in the *Typha latifolia* rhizome. Furthermore, it has been found that concentration of cadmium is highest in the stalk, while lead and manganese are found mostly in leaves of *Typha latifolia*. It is characteristic that there is no nickel in leaves, and that accumulations of zinc, iron and manganese are lowest in plant stalk.

When it is about *Phragmites communis,* it has been found that concentrations of cadmium, zinc, lead, iron and manganese are highest in rhizome. In case of copper and nickel, they accumulate mostly in the stalk.

Lemna is a genus of free-floating aquatic plants from the Lemnaceae family. These rapidly-growing plants have found uses as a model system for studies in community ecology, basic plant biology, in ecotoxicology, in production of biopharmaceuticals, and as a source of animal feeds for agriculture and aquaculture. The plant has a very simple structure that lacks obvious stems or leaves, with small plate-shaped structure floating on water surface. In addition to this it possesses high pollutant removal potential due to small size, fast growth, and easy to culture. It is also good bioindicator of heavy metal pollution.

The importance of the description of microscopic botanical aspects in order to help determine definitively the proper species of plant material being collected, harvested or processed, i.e., while the plant material is still in its non-extracted form. Microscopically descriptions can include the characterization of the histological structures, cells and cell contents visible only via light microscopy (LM) and scanning electron microscopy (SEM). The observation of cellular-level morphology or anatomy is a major aids. These characters are especially important for identification of out broken or powdered drugs, because in these cases most of the morphological diagnostic features are lost.

SEM is a powerful method for the investigation of surface structures of plants namely leaves, pollen grains and seeds. This technique provides a large depth of field, which means, the area of the sample that can be viewed in focus at the same time is actually quite large. SEM has also the advantage that the range of magnification is relatively wide allowing the investigator to easily focus in on an area of interest on a specimen that was initially scanned at a lower magnification. Furthermore, the tridimensional view images may be to an investigator it easier to interpret SEM images. In contrast to LM, which uses visible light as a source of illumination and optical (glass) lenses to magnify specimens in the range between approximately 10 to 1,000 times their original size, electron microscopy is operated in the vacuum and focuses the electron beam and magnifies images with the help of electromagnetic lenses (Flegler *et al.*, 1993).

The aim of the present study was to examine in vitro potential of *Lemna polyrrhiza L., and Lemna Triscula L.* to take up and accumulate Zinc, Cadmium and Nickel, to study the biochemical changes as well as response of the plant towards antioxidative enzyme stress exposed to the metal.

The objectives for the present study are as follows:

- Biodiversity study of wetlands in and around Baroda
- DNA Barcoding of these plant species.
- Establishment of phylogenetic relationship of related species.
- In vitro accumulation study of heavy metal ions (Zn, Ni and Cd) by selected plant species.
- Biochemical and Anatomical changes accompanying metal ion exposure to selected plant species.