REVIEW OF LITERATURE

Industrialization has caused a decisive bad effect on Earth climate, our natural resources, wild life, food and even the delicate ecosystem balance. Among various industries leather industry is one of the highly polluting industries.

2.1. TANNERY AND POLLUTION

Leather, the processed hide or skin of animals, is in use since pre-historic times. The tannery operation involves converting the raw skin into leather, a stable material that is resistant to decaying and wetting and is durable (Anonymous, 2010). According to Dixit (1995), the Indian tanning industry currently employs 80,200 people. Estimates of total production in a year are nearly 1500 million square feet of leather (Varadarjan and Krishnamoorthy, 1993). Indian Tanning industry, ranks amongst the five topmost export oriented industries of the country, is one of the oldest industries in India. The total export and Indian market of leather and leather products was estimated around 10 billion US dollar. There are about 2000 tanneries located in the States of Tamil Nadu, Andhra Pradesh, Bihar, Gujarat, Karnataka, Maharashtra, Punjab, Uttar Pradesh, Rajasthan and West Bengal spread all over India. The total processing capacity is about seven thousand tonnes per year (Anonymous, 2010).

Tanning is the process by which raw animal hides are converted into leather. During tanning the leather is made resistant to biological decay by stabilizing the collagen structure of the hide, using natural or synthetic chemicals (UNEP 1991). Bienkiewicz (1983) discussed the process of tanning in detail. The outline of basic techniques is given below (Fig.2.1).
During tanning process lime, sodium carbonate, chrome sulphate, sodium sulphate, sodium bicarbonate are used (Tudunwada et al., 2007). High amount of organic sulphide, nitrogenous compounds are used during leather finishings (Tadasse et al., 2007). It is reported that most common tanning agent used is chromium and vegetable tannins (Verma et al., 2008).

The environmental impact of the tanning industry is generally significant because of generation of wastes with plenty of organics salts and heavy metals (chromium compounds), both in solid and liquid form (Nazer et al., 2006). Due to worldwide mineral tanning, many metals specially Cr, Al, Zn, Pb, Ni, Cu, Fe, Mn, Na, Mg, Cl, Ca, K, sulphates, sulphides, etc. are expelled as hyper level pollutants. According to Gjerdåker (1998) the pollution load from the tanning activity is estimated to be 50% more in weight than the weight of the hides processed. Several of the sub-processes both organic and chemical contribute to Pollution. Since hides are organic material, large amounts of organic compounds are released when these are processed generating noticeable amount of water pollution. Around 175 different chemicals are in use for the tanning process in total.
Alarming level of heavy metals in the Hararibagh area of Dhaka city has been attributed to its nearly 185 Leather processing units (Anwar et al., 2006). Elevated levels of Fe and Cr has been reported in the agricultural soils irrigated with tannery effluents at Jajmau, Kanpur India. (Gupta et al., 2007). Substantial levels of toxic metals (Cr, Cd, Ni, and Pb) were detected in the leaves of maize fertilized with tanning residue (Souja et al., 2005).

Tannery industrial practices result in the discharge of effluents loaded with very high amounts of protein, chlorides, trivalent chromium, nitrogen, sulphate, COD, BOD, and suspended solids (Kadam, 1990). The released of untreated waste water from the tannery industry in river causes great damage to crop, soils and organisms (Alvarez bernal et al., 2006). Increase in the level of Cd, Cu, Cr, Pb, etc. was observed due to the tannery effluents water in crop soil (Gondek, 2006). The ground waters in the vicinity of many tanneries of Tamil Nadu have been found to be deteriorated in quality with TDS ranging from 1200 mg/l to 6000 mg/L. (Sastry and madhava krishna 1984).

The effluents rich in chromium, copper, zinc mainly and are released by tannery industries (Nouri et al., 2009). Gupta et al. (2007) had reported high amount of Cr, Fe in the agriculture fields due to tannery pollution.

### 2.2. Heavy Metal Uptake by Plants

Elements with metallic properties and an atomic number >20 are conventionally referred as heavy metals. Heavy metal like Cd, Cr, Cu, Hg, Pb, and Zn are natural components in soil (Lasat, 2000). Metals such as Zn, Cu, Mn, Ni, and Co are micronutrients necessary for plant growth while Cd, Pb, As and Hg have unknown biological function (Gaur and Adholeya, 2004).

Heavy metal polluted cultivation soils mainly contain chromium, copper, lead, mercury (Marques 2009). *Thlaspi caerulescens* is the plants which can accumulate up to 26,000 mg kg-1 Zn and up to 22% of soil exchangeable Cd from contaminated site (Brown et al., 1995 and Kochian, 1996). Henry (2000) reported up to 500 mg/l Pb uptake by *Brassica juncea*.

Metal hyper-accumulators are interesting model organisms to study for the development of a phytoremediation technology, the use of plants to remove pollutant metals from soils (Rai, 2012). Salt et al. 1995 had investigated the role of Ni tolerance
and transport in Ni hyperaccumulation by *Thlaspi goesingense*, using plant biomass production, evapotranspiration, and protoplast viability assays, and by following short- and long-tem uptake of Ni into roots and shoots. Ni tolerance, and not enhanced rates of Ni transport from root to shoot, is of primary importance in generating the hyper accumulator phenotype observed in hydroponically cultured *T. goesingense*.

Ghosh *et al.*, (2005) carried out pot experiment to study five weed species (*Ipomoea carnea, Dhatura innoxia, Phragmytes karka Cassia tora* and *Lantana camara*), with two accumulator plants (*Brassica juncea* and *Brassica campestris*) to assess Cr uptake in the range of 5 to 200 mg/kg soil. The results indicated that *P. karka* showed much greater tolerance to metals than other plants, though the uptake was low. It was more effective at translocating Cr from soil to plant shoot. The order of Cr extraction was *I. carnea > D. innoxia > C. tora > P. karka > B. juncea > L. camara > B. campestris*. Among the studied plants *I. Carnea* showed maximum chromium extraction and biomass growth, but the difference of shoot by root chromium concentration was least (Ghosh *et al.*, 2005).

Wu *et al.* (2005) examined the ability of 17 weed species and 5 weed species combinations to accumulate lead (Pb) from polluted soil in a greenhouse experiment. The weed species used were *Amaranthus viridis, A. spinosus, Avena fatua, Digitaria ciliaris, Echinochloa crus-galli var. mitis, Eleusine indica, Eragrostis pilosa, Gnaphalium affine, Ixeris chinensis, Lespedeza striata, Lolium perenne, Oxalis corniculata, Poa annua, Plantago virginica, Trifolium repens, Vicia cracca* and *Veronica didyma*.

The scientist had investigated metal hyper accumulators are plants that are capable of extracting metals from the soil and accumulating them to extraordinary concentrations (Chaney *et al.*, 1983). The highest contents of heavy metals were noted in *Lactuca serriola, Chenopodium album, Artemisia vulgaris* and *Atriplex nitens*. The concentration of Cd in the stems and leaves of *R. globosa* were greater than 100 mg/kg, under the conditions of the soils spiked with 25 and 50 mg/kg Cd.

Al-Zahrani and Abdulrahman (2014) investigated accumulation of Al, Ba, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Zn by five native plant species (*Dipterygium glaucum, Indigo feraspiniflora, Salsola kali, Suaeda aegyptiaca and Zygophyllum album*) growing at industrial area in Jeddah City and found that *Z. album* was an excellent candidate to remediate polluted soils.
Complex interactions of transport and chelating activities control the rates of metal uptake and storage (Salt et al., 1998). Plant metal accumulation determinants and selected engineering approaches to enhance metal accumulation capacity. Hyperaccumulating plants possess efficient mechanism for detoxification of these accumulated metals. According to Salt et al. (1998) these mechanisms include chelation, compartmentalization, biotransformations and cellular repair. Carbohydrates (such as melate, oxalate, citrate etc.) are commonly the major charge balancing anion present in the cell vacuoles of photosynthetic tissues. Several of these carbohydrates have been associated with high metal concentrations in plants (Gabbrielli et al., 1997; Homer et al., 1995). The tripeptide glutathione (GSH) is synthesized by gamma-glutamylcysteine synthetase (γ-ECS) and glutathione synthetase (GS). Increasing GSH synthesis is considered a mean to increase cellular defense against oxidation stress. Since GSH is precursor of phytochelatin (PC) over expression of γ-ECS or GS leads to high PC accumulation under metal exposure (Verbruggen et al., 2009).

To tide over the metal stress plants have evolved a number of mechanisms. These include the synthesis of the S-rich metal chelator glutathione (GSH) and phytochelatins (Hall, 2002, Gasic and Korban, 2006). GSH, the most abundant low molecular weight thiol compound in plants, play the main molecular weight thiol compound in plants, plays the main role in protecting plants from environment stresses. Plants secrete phytosidophores into the rhizosphere to chelate and solublise metals present in the soil. Metals enter the plants via root cells. Metals are first bound by the cell wall. Transport systems and intra cellular high affinity binding sites then mediate and drive uptake of metals across the plasma membrane. The membrane potential on the inner side of plasma membrane might exceed -200 m V in root epidermal cell and it provides a strong driving force for uptake of cations through secondary transporters (Hirsh et al., 1998).

Once inside the plant the insoluble metals from carbonates sulphate or phosphate precipitate immobilizing them in apoplastic (extra cellular) or simplistic (intra cellular) compartments (Raskin et al., 1998)

Tangahu et al. (2011) reviewed the heavy metals uptake by plants and depicted several factors which can affect the uptake mechanism of heavy metals as shown in Figure 2.2.
The uptake of a compound is affected by plant species characteristic (Burken and Schnoor, 1996). The amount of metal absorbed by plants is affected by the pH, organic matter, and the phosphorus content of the soil. According to Traunfeld and Clement (2001) adjustment of soil pH to a level of 6.5 to 7.0 with lime can reduce lead uptake by plants. Uptake of metals by plants is affected by the environmental conditions (Burken and Schnoor, 1996). The temperature affects growth substances and consequently roots length. Metal uptake by plants depends on the bioavailability of the metal in the water phase, which in turn depends on the retention time of the metal, as well as the interaction with other elements and substances in the water. Plants will affect the soil through their ability to lower the pH and oxygenate the sediment, which affects the availability of the metals (Fritioff and Greger, 2003), increasing the bioavailability of heavy metals by the addition of biodegradable physicochemical factors, such as chelating agents and micronutrients (Ginneken et al., 2007). Addition of chelating agents influences the bioavailability of heavy metals. The risk of increased leaching must be taken into account during use of synthetic chelating agents since the use of chelating agents in heavy-metal-contaminated soils could promote leaching of the contaminants into the soil (Ginneken et al., 2007).
Plant roots exude organic acids such as citrate and oxalate, which affect the bioavailability of metals (Seuntjens et al., 2004).

### 2.3. PHYTOREMEDIATION

Current methods for remediation of metal contaminated soils include soil removal and washing, physical stabilization, and/or the use of chemical amendments, all of which are expensive and disruptive, with an average cost of $404,700 per ha (Raskin et al., 1997). USEPA (2002) recommended excavation, capping, solidification and stabilization, nitrification, soil washing/acid extraction, soil flushing, phytoremediation, etc. as current remediation technologies for heavy metal-contaminated soil.

Phytoremediation includes any remediation method that uses plants to either remove pollutants or render them harmless in soil and water systems, and can be applied for both organic and inorganic pollutants present in soil, water, and air (Salt et al., 1998). Phytoremediation, also called green technology, can be applied to both organic and inorganic pollutants present in soil (solid substrate), water (liquid substrate) or the air (Gratao et al., 2005; Salt et al., 1998). Plants can be compared to solar driven pumps capable of extracting and concentrating certain elements from their environment (Salt et al., 1995b). This practice is gaining popularity because of its overall cost-effectiveness (Salt et al., 1998; Kabata-Pendias and Pendias, 2001).

![Fig. 2.3: Uptake mechanisms in phytoremediation technology](Source: ITRC, 2009)

Based on Figure 2.3, some certain essential processes involved in phytoremediation technology are phytostabilization and phytoextraction for inorganic...
contaminants, and phytotransformation / phytodegradation, rhizofiltration, and rhizodegradation for organic contaminants [USEPA, 2000; Prasad and Freitas, 2003].

a) **Phytoextraction** - the use of pollutant-accumulating plants capable to extract and translocate pollutants to the harvestable parts. It uses tolerant plants that concentrate soil contaminant in their above ground biomass, so that the contaminant-enriched biomass can be properly disposed (Kramer, 2005).

b) **Phytostabilization** – the use of pollutant-tolerant plants for mechanical stabilization of polluted land in order to prevent bulk erosion, reduce air-borne transport, and leaching of pollutants (Prasad and de Oliveira Freitas, 2003). Plants suitable for phytostabilization have extensive root system, provide good soil cover, possess tolerance to the contaminant metals and ideally immobilize the contaminant in the rhizosphere.

c) **Phytoimmobilisation**- the use of plants to decrease the mobility and bioavailability of pollutants by altering soil factors that lower pollutant mobility (formation of precipitates, insoluble compounds and sorption on roots).

d) **Phytovolatilization** - the use of plants to volatilize pollutants and has been demonstrated for selenium, mercury and arsenic. Plants extract volatile pollutants from the soil and biologically convert them to a gas which is released via transpiration from the foliage (Ghosh and Singh, 2005a; Ghosh and Singh, 2005b; Raskin et al., 1997).

e) **Phytodegradation**: the use of plants to breakdown of organic contaminants to simpler molecules which are stored in the plant tissue (Ghosh and Singh, 2005b).

f) **Rhizofiltration**: the approach of using hydroponically cultivated plant roots to remediate contaminated water through absorption, concentration, and precipitation of pollutants (Dushenkov et al., 1995).

Tangahu *et al.* (2011) reviewed the heavy metals (As, Pb, and Hg) uptake by plants through Phytoremediation technologies and explained the mechanisms of phytoextraction, phytostabilisation, rhizofiltration, and phytovolatilization as shown in Figure 2.4.
Phytoextraction is the process that extracts both metallic and organic constituents from soil by direct uptake into plants and then translocation these constituents to aboveground biomass and this is successful when (hyper) accumulating plants are used. According to Brooks (1998) the hyper-accumulators are exceeding the hyper-accumulation threshold i.e. Concentration 100 times higher than in normal plants for each metal of interest, i.e. (mg kg⁻¹): 100 Cd; 1000 As, Ni; 10000 Zn, Mn, etc. Among the best studied hyper-accumulators (< 0.2% angiosperms) the mention may be made of Brassica juncea, Brassica oleracea, Berkeyia coddii, Allysum bertolonii, Thlaspi caerulescens and Thlaspi goesingense. More than 400 plant species have been identified as metal hyper-accumulators (Wu et al., 2010; Mudgal et al., 2010).

Zhuang et al. (2007) had investigated phytoremediation potential for Pb, Zn, and Cd by high biomass plants like Vertiveria zizanioides, Dianthus chinensis, Rumex K-I (Rumex upatentia x R. timschmicus), Rumex crispus, and Rumex acetosa. It was
found that phytoremediation for Cr can be done by *Zea mays*, *Sorghum bicolor*, *Helianthus annuus* (Abou-Shanab et al., 2004). Chatterjee et al. (2006) investigated phytoremediation potential of *Cynodon dactylon*, *Colocasia antiquorum*, *Cyperus rotundus*, *Eichhornia crassipes*, *Ipomoea aquatica*, *Wolffia arrhiza*, *Pista stratotes* for Cr, Cu, Pb, Zn, Mn, Mg, and Fe. Significant phytoextraction capacity was seen in *Chenopodium album* for Fe, Mn, Zn, Cr, Pb, Ni and Cd (Gupta and Sinha 2007). *Typha angustifolia* shown efficient phytoremediation potential for Cd, Pb, Cr, Ni, Zn and Cu. (Demirezen and Aksoy, 2004). Even the aquatic weed like *Eichhornia crassipes* is reported to be potential phytoremediator for heavy metals in the polluted aquatic bodies. (Tiwari et al., 2007).

Lone et al. (2008) attempted to provide a brief review on recent progresses in research and practical applications of phytoremediation for soil and water resources. More than 400 plant species have been identified to have potential for soil and water remediation. Among them, *Thlaspi*, *Brassica*, *Sedum alfredii* and *Arabidopsis* species have been mostly studied. Plants of families *Brassicaceae*, *Fabaceae*, *Euphorbiaceae*, *Asteraceae*, *Lamiaceae*, and *Scrophulariaceae* are know a potential phytoremediators (Salt et al. 1998, Dushenkov 2003).

Kumar et al. (1995) carried out investigations on high biomass accumulating fast growing members of *Brassicaceae* like Indian mustard (*B. juncea*), black mustard (*B. nigra*), turnip (*B. campestris*), rape (*B. napus*), Kale (*B. oleracea*). Among these *B. juncea* showed a strong ability to accumulate and translocate Cu, Cr, Cd, Ni, Pb and Zn to shoots.

According to Ginocchio and Backer (2004) numerous plants with hyper-accumulation potential or tolerance to metals have been discovered in Brazil, Chile, Cuba, the Dominican Republic and Venezuela and include Ni (89%), Cu (5%) and As (3%) hyper-accumulators. Many hyper-accumulators have been suggested for phytoextraction; however, most of these plants achieve low shoot biomass. Therefore, the use of trees in phytoextraction has been suggested and recently reviewed by Pulford and Dickinson (2005). *Alnus incana*, *Betula pendula*, *Fraxinium excelsior*, *Sorbus mongeotii*, *Salix* and *Populus* species are some of the trees reported to grow on contaminated lands. Based on screenings, it has been found that *Salix* (willows) and *Populus* (poplars) are capable of accumulating metals, especially Cd and Zn in substantially large amounts in their leaves. Large metal concentrations (300 mg Cd
kg-1) were found in leaves of *Salix smithiana* BOKU 03 CZ-001, *S. purpurea* BOKU 05 CZ-001 and *S. caprea* BOKU 01 AT-004 clones growing on contaminated soils (Cd total 30 mg/kg) and in shoots of *S. viminalis* (2695 mg Zn/kg) grown on an acidic soil (Zn total 1158 mg/kg) (Hammer *et al*., 2003).

Phytoremediation is the best approach to remove contaminants primarily from soil and isolate them without destroying the soil structure and fertility (Gosh and Singh, 2005). Another advantage of phytoextraction is that during this practice, the soil erosion and leaching are minimized (Jadia and Fulekar, 2009).

Mobility and availability of heavy metal in soil and plant is major factor which affect the phytoextraction potential. The efficiency of phytoremediation can be quantified by calculating Biological Concentration Factor (BCF), Biological Accumulation Coefficient (BAC) and Translocation factor (TF). All these parameters have been studied in the *in vivo* evaluation of all the seven species of weeds (polluted genotype). BCF was calculated as metal concentration ratio of plant roots to soil (Yoon *et al*., 2006). Biological Accumulation Coefficient (BAC) was calculated as ratio of heavy metal in shoots to that in soil (Li *et al*., 2007; Cui *et al*., 2007). The TF expresses the capacity of a plant to store the metals in its upper part and is often calculated to quantify the efficiency of phytoremediation. This is defined as the ratio of metal concentration in the upper part to that in the roots (Chakroun *et al*., 2010). Translocation Factor (TF) was described as ratio of heavy metals in plant shoot to that in plant root (Cui *et al*., 2007; Li *et al*., 2007). The translocation factor indicates the capability of the plant in translocation the accumulated metal from its roots to shoots. A translocation factor value greater than 1 indicates the translocation of the metal from root to above-ground part (Jamil *et al*., 2009).

The extent of metal removal is most important factor affecting the selection of phytoremediating species. The efficiency of phytoextraction by a given species depends on two key factors biomass and metal accumulation factor (Blaylock *et al*., 1997). Metal hyper-accumulators and non-accumulator species have been evaluated for their selection as remediative species. The use of hyper-accumulator plants may be limited by small size and slow growth. The non-accumulator species having low potential for metal uptake may compensate it by the production of significant biomass (Ebbs *et al*., 1997). However, at many sites metal contamination is high enough to cause toxicity to plant species and causes significant biomass reduction. The plant
species may accumulate high level of one metal but may be susceptible to another metal rendering it not suitable for metal cleanup operation (Hinesly et al., 1978). According to Chaney et al. (1999) several maize inbred lines identified for their potential to accumulate high levels of Cd could not be used to cleanup soils at the normal Zn: Cd ratio of 100:1.

Plant biomass, bioconcentration factor and soil mass are the three key variables that define phytoremediation potential of a given species (Zhao et al., 2003). Phytoremediation is essentially an agronomic approach and its success depends ultimately on agronomic practices applied at the site. The importance of employing effective agronomic practices has been discussed by Chaney et al. (1999). Practices have been developed to increase the potential of common non-accumulator plants for metal phytoextraction. Particularly, the uptake-inducing properties of synthetic chelates (Huang and Cunningham, 1996; Blaylock et al., 1997), the addition of organic fertilizers, lime etc. Phosphorus is a major nutrient, and plants respond favorably to the application of P fertilizer by increasing biomass production but can inhibit the uptake of some major metal (Chaney et al., 2000).

Phytoremediation of cadmium, lead zinc by Brassica Junceae L. Czern and Coss. Brassica juncea has been found to have high potential to remediate Cd, Pb and Zn from aquatic environment with up to a maximum concentration of 50µg/ml. this plant can therefore be grown in aquatic environment that are contaminated with heavy metals (cadmium, lead, zinc), after which the plant biomass can be harvested and burned to ash to recover the metals or to be disposed of appropriately and safely (Salt et al. 2009).

Phytoremediation of Cadmium Contaminated Soil by Sunflower was investigated by Abdullah et al., 2013. Sheoran et al. (2012) investigated Phytoremediation of Metal Contaminated Mining Sites. Phytoremediation using Phragmites australis roots of polluted water with metallic trace elements was investigated by Meriem et al. (2013)

Swapna et al. (2014) studied the accumulation pattern of heavy metals Al, Cd, Fe, Hg, Cr, Cu, Pb, Ni and Zn in Chromolaena odorata and observed significant variations in the quantity of accumulation as well as distribution among plant parts like root, stem and leaf. Maximum quantity of each metal was accumulated in the root as compared to stem and leaf.
Archaya et al. (2014) reviewed the advances in phytoremediation of pesticide polluted soils and advocated phytoremediation as a promising technology for cleanup of hazardous organic and inorganic pollutants, which include agro-chemicals polycyclic aromatic hydrocarbons and polychlorinated biphenyls.

Phytoremediation as a ecological solution to heavy metal polluted water and Evaluation of Plant Removal Ability was investigated by Jatin et al., (2013). Kamaruzzaman et al., (2011) investigated accumulation and distribution of Lead and Copper in *Avicennia marina* and *Rhizophora apiculata*. Mahdavi et al. (2012) investigated Pb and Cd accumulation in *Avicennia marina*. Karimi (2013) carried out to investigate the potential of alfalfa (*Medicago sativa*) and sorghum (*Sorghum bicolor*) for phytoremediation of soil contaminated with chromium. The study revealed that both are effective accumulator of chromium, but the potential of alfalfa was more than sorghum for phytoremediation of chromium from polluted soils.

Mojiri (2012) investigated phytoremediation of heavy metals from municipal wastewater by *Typha domingensis*. These results showed that most metal removal from wastewater by *Typha domingensis* was after 48 h and in order of Fe>Mn>Zn>Ni>Cd. Singh et al. (2011) studied the phytoremediation of lead from wastewater using aquatic plants.


The advantages of Phytoremediation technology are i) It is aesthetically pleasing , solar energy driven clean up technology ,ii) there is minimal environment disruption, iii) it preserves top soil due to *in situ* nature, iv) it is most useful at sites
with shallow, low level contaminants, v) useful in broad range of contaminants, vi) it is inexpensive (60-80%) less costly than conventional physio-chemical methods. However, it is time consuming process and may take several growing seasons to clean up a site and disposal of metal rich plant material is also problematic.

Identification of candidates for removal of heavy metals by phytoremediation is still at its preliminary stage. Keeping in view the presence of heavy metals in the soils of the study area in Kapurthala-Jalandhar belt of Punjab, the present problem was taken up to identify the species suitable for remediation of the soil.