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# THE ROLE OF PHYTOREMEDIATION IN THE SOIL

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***Abstract:*** *Pollution of our environment with heavy metals as a result of industrialization and use of agrochemicals to boost agricultural production is continuing unabated and is affecting animal and human health. As system of intensive agriculture is developing near the cities and making use of high doses of fertilizers and sewage sludge, Government and Public Opinion in world has started questioning the possible impact of these agro-chemicals and agricultural practices on the three components of our "Biotope": Soil, air and water (Environment). Soils generally contain low levels of toxic heavy metals viz., cadmium (Cd), Mercury (Hg), Lead (Pb) and Nickel(Ni) but considerable amounts of the elements can be introduced into soils via anthropogenic pathways such as dumping industrial effluent and agricultural application of sewage sludge, use of inorganic and organic agro-chemicals, application of commercial fertilizers. In addition to above mentioned key sources of heavy metals contamination in soil, the products used in our modern society are also too toxic to be disposed off without particular treatment. Not only the industries generate such kind of poisonous and hazardous waste but also many household products fall under this category. If not disposed away correctly, some cleaners, solvents, pesticides, paints in the form of sewage water can contaminate the agricultural soil in the vicinity of cities, leak into the underground water or contaminate the irrigation sources resulting in tremendous risks for the safety and health of human. In the respect of our environment, the basic recommendations of (reduce, recycle, reuse) should prevail as a prevention, instead of curing. As good as it can be applied, this principle cannot totally avoid generation of hazardous waste and therefore, long term solution must be developed. For sustainable clean ups of environment from these hazardous wastes and heavy metals, techniques and strategies includes phytoremediation. It is a three step process involving high uptake of heavy metals by roots, transportation to shoot and sequestration (distribution) of metal within the shoot. Hyper accumulator plant species exhibit the shoot/root ratio of metal content greater than 1, which reflect that specific internal system pumps metals from plant roots to shoot tissue (8).The chelator-assisted phytoremediation studies has been carried out on the phytoremediation of Cd, Ni, Pb and Hg polluted soils by Brassica species under screen house condition in spiked soils in the Department of Soil Science, CCS Haryana Agricultural University Hisar-India.*

***Keywords:*** *chelating agents, heavy metals, hyperaccumulattor,microbes, phytoremediation*

## INTRODUCTION

The use of specially selected and genetically engineered metal accumulating plants for environmental clean-up is an emerging frontline technology called "Phytoremediation" which describes a system wherein plants in association with soil organisms can remove or transform contaminants into harmless and often valuable forms. [9]. Heavy metal pollution of soils in all over the world is an alarming and catastrophic situation requiring urgent rejuvenation and decontamination. This has forced the researchers to think of using plants for cleaning of their own support system.

Local metal enrichments are either natural or result from human activities, such as smelting, mining, processing, agricultural and waste disposal technologies. The release of metals by industrial activities at some extent has been limited in Europe. As Indian and Middle East countries scenario is concerned, due to rapid industrial development and urbanization during the last two decades, disposal of industrial effluents, electronic waste has become a serious problem because they contain a large amount of heavy metals (Cd, Ni, Pb, Cr and Se). In addition to this, hospital disposal, city waste and lack of facilities of sewer water treatment (except in few metropolitan cities where treatment facilities are available) adds a large amount of heavy metals to soil. Farmers grow vegetables in sewer water irrigated areas in lure of saving some fertilizer, as sewer water and effluents are rich in nitrates and phosphates. Also for saving transportation costs, farmers grow vegetables in areas around the big cities, which are highly polluted by city waste, sewer water, electronic waste and hospital disposal. Use of agrochemicals viz. pesticides, herbicides and insecticides which contain heavy metals (Hg, As and Pb) at large scale make the situation more dangerous. Above mentioned points clearly indicate that vegetable growing areas are highly contaminated in India and in other Middle East countries also. Vegetables bring heavy metals directly in food chain and cause serious health hazards to human beings. So it is a matter of serious concern that there is a very emphatic need to remediate these vegetable growing areas with phytoremediation technique.

In view of this, the research project was planned with the following objectives:

1. Relative efficiency of Brassica species, and Maize to accumulate heavy metals (Cd, Hg, Pb and Ni) with and without application of chelating agents (EDTA, NTA) in soil.
2. To investigate the accumulation factor (AF) for different heavy metals in some hyper accumulator plant genotypes.
3. To investigate the heavy metals content and speciation of Cd, Hg, Pb and Ni in sewage sludge applied soils and decontamination with introducing hyper accumulator crops in local crop-rotation.
4. The heavy metal tolerant bacteria shall be identified, characterized and utilized for biosorption of heavy metals in the industrial effluents and waste water.

## MATERIALS AND METHODS

This study included green house/field trials as well as laboratory experiments. The physico-chemical characteristics of soils were determined using standard procedures and heavy metals have been determined by ICP.

1. Homogenous (metal spiked) soils for different heavy metals (Cd, Hg, Pb and Ni) prepared in bulk and different hyper accumulator test crops species tested for accumulation of metals.
2. Similarly sewage sludge soils from areas in vicinity of urban areas which used for intensive cultivation had been collected and repetition of above mentioned hyper accumulator for decontamination.
3. Effect of different chelating agents/rhizotrons to boost phytoremediation observed and soil application in solution form pre and post germination of crop were performed.
4. Isolation and characterization of heavy metal tolerant bacteria and their utilization in bioremediation of soil and waste water.

**Screen house experiments:** The screen house experiment consists of the following organic amendments and microbial biomix treatment combinations.

A. Metal-enriched soil as such ($Cd_{50}$)
B. Metal-enriched soil + FYM @ 2% ($Cd_{50}$+ FYM)
C. Metal-enriched soil + Vermi Compost @ 2% ($Cd_{50}$ +VC)
D. Metal-enriched soil + Microbes *(Azotobacter spp-Mac-27*)
E. Metal-enriched soil + Microbes *(Pseudomonas spp-P-36*)
F. Specific metal enrichment in incubation tests A-E are: i) $Cd_{50}$ ii) $Ni_{200}$ and iii) $Pb_{400}$

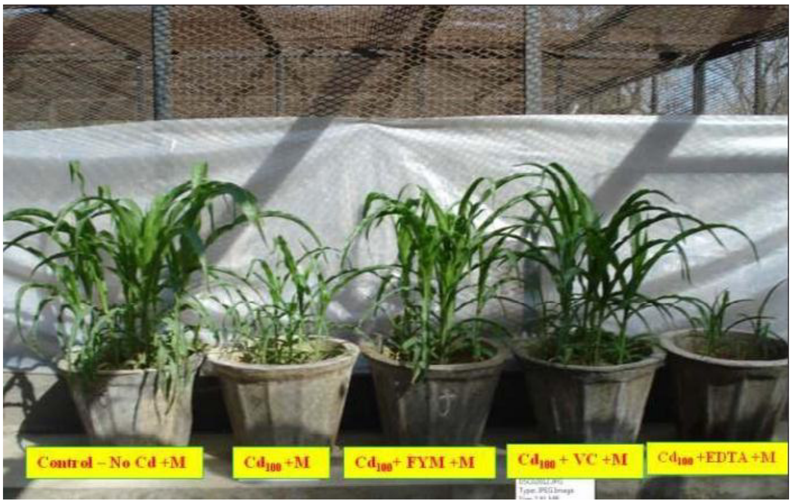
## Test crops

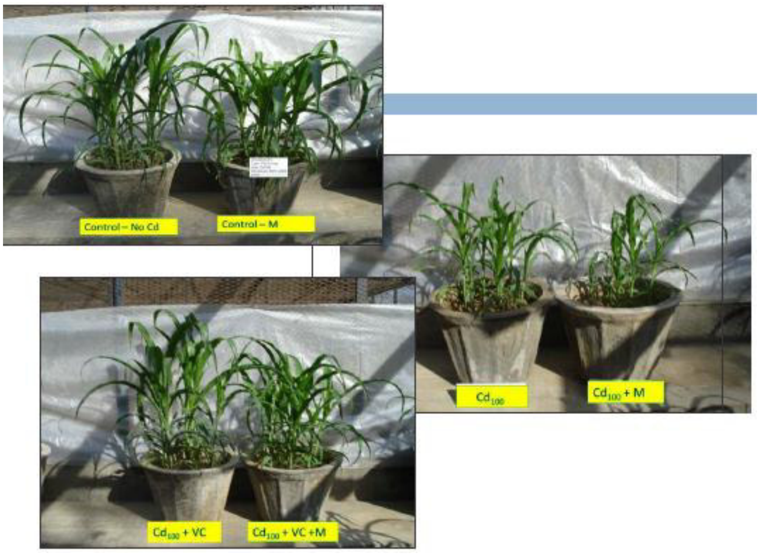
1. Indian mustard *(Brassica juncea* L*),*
2. Sunflower *(Helianthus annuus* L.*),*
3. Maize (*Zeya mays*)

Plant samples were digested in cc.$HNO_3$ + cc.$H_2O_2$ and the heavy metal contents measured by ICP-AES techniques.

## RESULT AND DISCUSSION

Reports of infamous itai-itai and minamata diseases from Japan due to excessive dietary intake of cadmium and mercury by human beings [2] are the specimens of heavy metals pollution. Health hazards associated with lethal intake of Cd included renal (kidney) damage, anemia, hypertension, and liver damage [15]. Mercury levels in human beings have been directly related to in gestation of fish reared on Hg-polluted aquaculture, although agricultural use of mercurial fungicides can also cause entry of Hg into human system through crop plants [2]. Principal anthropogenic sources of Pb and As are use of leaded gasoline and spraying of arsenic pesticides. Encephalopathy (damage to brain), neurophysical deficit in children and metabolic disorder are caused by the lead intake by human and animals.*Thlaspi* have been grown in soils contaminated by smelter fumes.




These contaminated soils are virtually barren. Accumulating more than 30,000 mg/kg (about 3%) zinc in their tissues, the *Thlaspi* plants grown on this site, could be harvested to remove large quantities of the metals from the soil. This and another phytoremediation technologies for metals (e.g. the bio-reduction of Cr and Se ions) hold promise for cleaning up or making them less harmless the badly contaminated soils.

The survey of literature suggests that some crop plants like Indian mustard and maize possess the ability to hyperaccumulate the toxic heavy metals [(6]; [18]; [13]. Micro-organisms produce a wide range of different chelating agents like siderophores which make metals more available for plant uptake and therefore may be useful for remediation application [7]; [11]. Plant growth can also be promoted by microorganisms that produce growth factors, protect plants from the toxic metals or make soil micronutrients and metals bioavailable to plants [23].

Use of synthetic chelating agents (like EDTA, NTA etc.) is known to promote metal extractability, increased availability to plants and transformation to other forms/complexes [12]; [10]; [17]. The C-EDTA-4 with a release period of 80 days proved to be the best in decreasing Pb and EDTA concentrations in soil solution and increasing Pb accumulation in sorghum shoots compared to the direct application of EDTA. [22].

## HYPERACCUMULATOR

Phytoremediation is a new technique employed for the remediation of contaminated soils. *Brassica juncea* (Indian mustard) accumulates high amounts of Pb, Cd, Cu, Ni and Zn in shoots. Metal uptake and accumulation in shoots of *B. juncea* is shown to be enhanced by application of chelators [1]; [16]; [21]. Phytoextraction by using hyper accumulator plants may provide an effective and in situ way of removing heavy metals from contaminated soils by metal accumulating plants, are used to transport and concentrate metals from the soils into the harvestable parts of roots and above ground shoot. *Brassicaceae* and *Compositae* families are capable of having high levels of heavy metals in their above-ground plant organs. In many soils affected by mining, smelting, industrial processes and application of sewage sludge contaminated with single metal is most uncommon but with several metals usually occurring at high content. Nickel is often found in high content in polluted soils with Cd and Zn, and the sensitivity of highly Ni/Cd/Zn tolerant hyper accumulators has not been documented. The efficiency of metal uptake by hyper accumulating species is poorly defined in the situation where several metals are present at high concentrations. The term hyper accumulator has come to mean a plant capable of taking up concentrations of heavy metals approximately 100 times greater than normal plant species [3]. It has been suggested that highly specialized plants that have evolved the ability to accumulate and tolerate very high concentrations of metals from soils may provide the basis for remediation of heavy metals. Hyper accumulator species have been found that contain > $10^3$ mg Zn $kg^{-1}$ and >100 mg Cd $kg^{-1}$ shoot biomass [4]. Before, phytoremediation can be effectively exploited on contaminated soils, better agronomical practices for metal uptake by hyper accumulator species is necessary.

The use of plant roots to remove heavy metals from water is also an emerging environmental clean-up technology. Roots of many hydroponically grown terrestrial plants, e.g., Indian mustard, sunflower and various grasses, effectively removed the toxic metals such as $Pb^{2+}$, $Cd^{2+}$and $Ni^{2+}$from aqueous solutions. Roots of *B. juncea* concentrate these metals 131-563 times (on a dry weight basis) above initial solution concentration of Pb/Cd. The uptake was based on tissue absorption and on root-mediated Pb/Cd precipitation in the form of insoluble inorganic compounds, mainly leads phosphate/Cd phosphate.

Heavy metals uptake in Indian mustard is proportional to the amount of metals contained in a measurable fraction of the soil solution and plant capable to tolerate higher levels of heavy metals in soils and in shoot tissue also. The shoot/root ratios for Cd, Ni, Pb and Hg are much higher for these *Brassica* species. A greater portion of total metal in the plant is therefore, sequestered in harvestable tissues, [17].

Threshold for cadmium hyper accumulator has been set at 100 µ g $g^{-1}$ D.M. [4]. The hyper accumulator plant (*T. caerrulscens*) could take up 0.16 kg $ha^{-1}$ Cd and 0.25 kg $ha^{-1}$ Ni in a single harvest of crop. The *T. caerulescens* cultivation in contaminated soil, assuming a constant rate of Cd uptake with 0.40 mg Cd $kg^{-1}$D.M. removed in 5 weeks, all the cadmium content in soil would be removed after 15 years and the *C. halleri* accumulated 60 g Cd $ha^{-1}$in a single harvest of crop. Development of phytoremediation must be a process of matching the plants to the particular situation. High biomass crops such as *Brassica* species may be useful, especially with addition of metal chelating or acidifying compounds which would boost the metal concentrations in the crops by desorbing the metals from the soil and overcoming any limitation to their transport to the site of uptake in the roots and translocation to the shoots. In India considerable effort is being devoted to identifying indigenous plant species that can be used to remediate pollutants such as pharmaceutical wastes, arsenic, fly ash, and metals. It is fairly low in cost, does not require extensive equipment, and is appropriate not only for India, Iran, Pakistan, Bangladesh, and other Asian nations, but also for advanced nations [14].

## FERTILIZER IMPACT

The spontaneous, increase of heavy metals in the agricultural soils with the use of commercial fertilizers is also another problem for farmers, The phosphogypsum (industrial byproduct) is contaminated with 3.4 to 14.0 mg Cd $kg^{-1}$ and gypsum (natural) from 0.12 to 5.0.

Agricultural lime (industrial byproduct) 14.0 mg Cd $kg^{-1}$ Zinc sulphate 24.9 mg Cd $kg^{-1}$) and copper sulphate 17.2 mg Cd $kg^{-1}$fertilizer. The rock phosphate (Parent material for P fertilizer) having Cd, Hg, Ni and Pb 90, 2.1, 51 and 51 mg $kg^{-1}$D.M., respectively. A source of rock phosphate also contaminated with heavy metals. USSR (Kola) having 1.0 mg Cd $kg^{-1}$P and 0.20 mg Cd $kg^{-1}$D.W.,


Plate: Comparative toxicity of Cd, Ni and Pb in Indian Mustard (Panwar and Grewal, 2013)

Naura rock phosphate 611 mg Cd $kg^{-1}$P and 100 mg Cd $kg^{-1}$and China (Yunan) rock phosphate 35.0 mg Cd $kg^{-1}$P and 5.0 mg Cd $kg^{-1}$D.W. High Cd contents was found in fertilized soils (0.01-0.73 mg Cd $kg^{-1}$soil) as compared to unfertilized soils (0.01-0.29 mg Cd $kg^{-1}$soils. In India (1998-99), the consumption of P fertilizer was 95.3 lakh tones (58.1 DAP and 37.2 SSP) whereas, production of $P_2O_5$ was 3170 thousand tones).

Soil fertility status for phosphorus in all over India (17 districts is high P, 184 districts medium and 170 districts low in P) hence, major parts of agricultural soils fall in the medium to low category which need P fertilization for production of high yields.

## EFFECT OF CHEMICALS

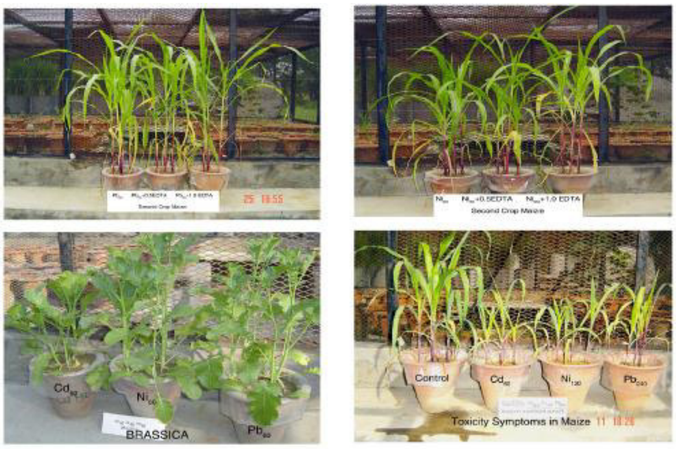
### Organic Chelates, Microbes and Bacteria

Huang *et al*. [10] indicated that EDTA was the best chelating agent for mobilizing Pb from the soil particles. Gradual acidification to release more metals from the soil matrix, the EDTA (Ethylene diamine tetracetic acid) is one of the most promising chelator. The ability of this chelant to bind the metals ions in extremely stable complexes can be utilized not only for desorption of adsorbed ions, but for the dissolution of insoluble metal compounds as well.

Panwar *et al* [19] reported that with maize (*Zea mays)* grown on artificially spiked soil (100 μ g Cd $g^{-1}$) with EDTA (2 mmol $kg^{-1}$ in 5 split doses), FYM, Vermicompost (VC) and Microbial inoculants (*Azotobacter* and *Pseudomonas*). The growth of maize was better in farm yard manure (FYM) or vermicompost (VC) amended soil as compared to no-Cd- enriched soil. The growth was little suppressed in EDTA applied soil whereas it was better in bioinoculants treated soil.

The application of FYM and Vermicompost (VC) increased the dry matter yield of maize either alone or in combination with bioinoculants. The application of EDTA caused a significant decrease in biomass of crop plants. Application of bioinoculants increased the dry matter yield of both root and shoot but not significantly because bioinoculants show greater sensitivity towards cadmium.

Cadmium concentration was observed maximum under EDTA with bioinoculants treatments, however Cd uptake was maximum in Vermicompost with inoculates treatments. The Cd concentration in shoot over $Cd_{100}$ treatment increased in $Cd_{EDTA}$ followed by $Cd_{VC}$ and $Cd_{FYM}$ in the absence of microbial inoculates. The corresponding trend in the presence of microbial inoculates were as same, respectively. Similar trend was also observed in root as in order $Cd_{EDTA+M}$> $Cd_{VC+M}$>$_{CdFYM+M}$> $Cd_{100+M}$.




**Effect of chelating agent (EDTA) on crops**

The remarkable root and shoot Cd content increase was observed with microbial inoculates in $Cd_{EDTA+M}$ treatment over $Cd_{EDTA}$ treatment. The FYM, Vermicompost and EDTA also increased Cd uptake significantly with and without microbial inoculates in root shoot biomass.
The results indicated that Vermicompost in combination with bioinoculants is better and proven implication for the phytoremediation of Cd by maize crops from Cd-polluted soil being higher uptake value in shoot in $Cd_{VC+M}$ followed with $Cd_{EDTA+M}$, $Cd_{FYM+M}$ and $Cd_{100+M}$ treatment combinations. Recently, the native flora and microbes from various research laboratories, the algae, cyanobacteria, vascular plants, and aquatic macrophytes have been used extensively in poly houses and field conditions [l4].
Bhupender and Sharma [5] conducted a study for identifying heavy metal tolerant bacteria in soil samples obtained from three different sites in and around Solan city of Himachal Pradesh. The samples included industrial effluents from manufacturing unit of batteries and invertors at Oachhghat, metal dumping site at Chambaghat (Salogra) and domestic waste contaminated site at Solan city.
From each of the three sites 8, 8 and 6 soil samples respectively, the sites of the collection in each category were located 5-10 meters apart.
The serial tenfold dilutions of the soil samples upto $10^{-6}$ were prepared. The highest addition of the sample dilution ($10^{-6}$) was inoculated into nutrient agar for isolation of bacteria. Colonies with different morphological features from the mixed cultures were subcultured in order to obtain pure colonies. These colonies were then grown on nutrient agar containing different molarities of salts of heavy metals (Zn, Cd, Pb and Co). The highest concentration at which the growth was observed is as follows: Zn-8mM, Cd-4mM, Pb-6mMand Co-4mM.
The bacteria having multiple tolerances were identified as *Comamonas, Pseudomonas, Staphylococcus nepalensis, Bacillus licheniformis, B. cereus, B. subtilis and Micrococcus spp.* on the basis of colony morphology, microscopic examination and biochemical tests. One sample each of *Comamona testesteroni, Staphylococcus sonerensis and Bacillus licheniformis* were identified on the basis of partial nucleotide sequencing of amplicons of 16S rRNA.
The tolerant isolates were further subjected to *in vitro* antibiotic cultural sensitivity assay and were found resistant to multiple antibiotics. The antibiotic resistance may be correlated to heavy metal tolerance. The bacteria might be of human origin.
However further studies are required to establish these facts. The study is of significance in that such bacteria can be utilized for phyto-bioremediation of heavy metals in the environment.

**Hyperaccumulator Crop Species:**

1. Indian mustard
2. Maize
3. Indian cotton
4. Castor
5. Sunflower
6. Indian mustard

| Trees | Perennial grass | Crops |
|---|---|---|
| Pine | *Arrhenathermelatius* | Indian mustard |
| Oak | Salix clones | Maize |
| Hybrid Poplar | | Indian cotton |
| $NM_6$ (Nigra x maximo-wiczil | | Castor |
| | | Sunflower |

**Induced heavy metals with synthetic, organic complexing agents, microbial and bacterial activity:**

1. EDTA, NTA, FYM, Compost, Vermicompost
2. EDDHA (Fe-chelator)
3. Rhizotrons
4. Azotobacter
5. Bacteria/Microbes

## REMEDIATION TIME SPANE:

AF = Plant accumulation (mg $kg^{-1}$ D.M.)

Metal content (mg $kg^{-1}$ soil)

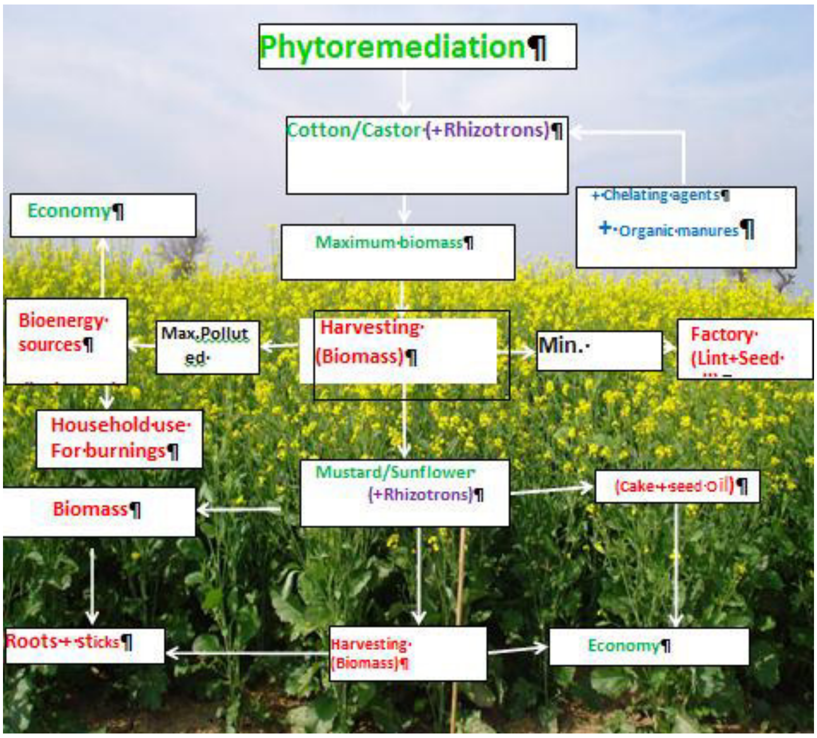
RF= Plant canopy,$ha^{-1}$ x CH

Metal quantity,$ha^{-1}$

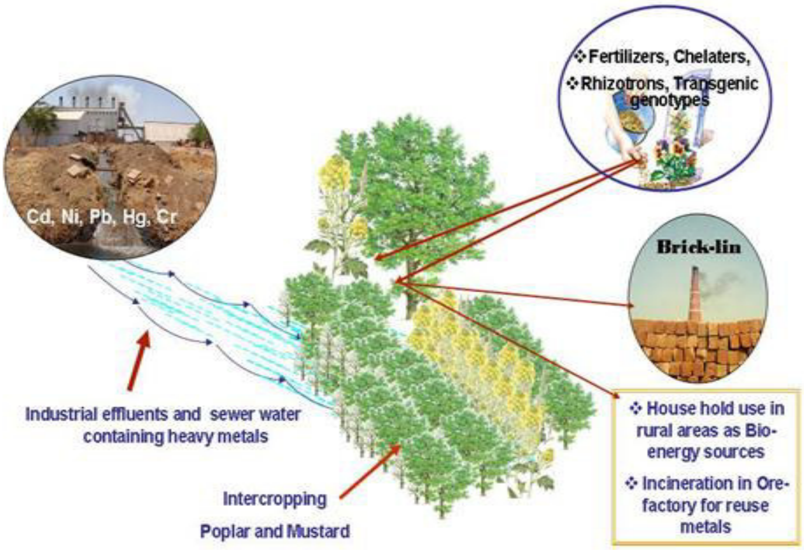
Abbreviation: AF = Accumulation factor, RF = Remediation factor, CH = Crop harvest

## CONCLUSION

i. Brassica species and maize had been proved as hyperaccumulators in Indian ecosystem
ii. Organic and synthetic chelating agents increased uptake of metals and biomass of crops




Proposed cropping model for moderately heavy metal contaminated soils




Schematic diagram of phytoremediation

Panwar(2010) CCS HAU, Hisar, India

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