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Integrated approach for selective utilization of phytobiomass: efficient phytoremediation

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ABSTRACT

Natural resources, especially soil and water bodies which are near to mining area or industrial area are most polluted with toxic heavy metals since industrial revolution. The persistent heavy metals contaminations becomea threat to 'Man and Biosphere'. Implementing sustainable practices such as phytoremediation contributes to mitigating the ongoing threat of heavy metals by reclaiming these contaminants from polluted soil. Recently, naturally occurring hyperaccumulator, tolerant species and transgenic plants are used for heavy-metal extraction. Extensive research is focusing on phytoremediation using plants like *Pteris vittata*, *Ricinus communis*, *Jatropha curcas*, and *Cannabis sativa,* Brassicaceae, Asteraceae, to extract heavy metals from the soil. Choosing an integrated system is crucial, where plant species act as hyperaccumulators and their biomass is utilized for purposes like biofumigation, biofortification and bioenergy production. Despite of certain limitations, the phytoremediation is one of the most efficient and cost-effective technology for reclamation of heavymetal from soil. Thus, the review mainly focuses on some known hyperaccumulator selectively utilized in field of biocides, biogas and nutrient enrichment of crops and biochar production for efficient phytoremediation.

1. Introduction

In recent years, it has been a great concern on ecological and global public health which is associated with environmental contamination by heavy metals due to rapid increase in industrialization, agricultural practices and the mining activities. (Sandeep *et al.*, 2019; Wang *et al.*, 2017). Heavymetals are the metallic element characterised by high densities to water (atomic mass greater than 20) and due to their persistence nature, they are considered as global pollutants. There are two types of metals either essential for plant or non-essential. Elements including cobalt (Co), copper (Cu), iron (Fe), nickel (Ni), manganese (Mn) and zinc (Zn) are basic heavy metals that are considered as essential micronutrients but can become toxic when present in excessive amount. On contrary, cadmium (Cd), mercury (Hg) and lead (Pb), chromium (Cr) are non-essential heavy metal that have adverse effect on living organisms even at low concentration and have no role in plant metabolism (Sandeep *et al.*, 2019). Various sources, such as industrial waste, surface runoff, human activities, mining, fossil fuel combustion, automobile exhaust, industrial processes and the cultivation of vegetables in contaminated areas, collectively contribute to the deposition of heavy metals in the biosphere.

The metal pollution has very much impact on biological system as it does not undergo the process of biodegradation. Accumulation of these metals in food chain result in biomagnification and it adversely affects the ecosystem. Heavy metal not only exert a significant impact on fauna and flora but also have consequences on the soil nutrient profile, (Sandeep *et al.*, 2019). Further more, it also inhibits photosynthesis, enzymatic activity and cellular integrity, indirectly reduced the growth and development of plant. People are encountering heavy metal by inhalation,

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$^{\Psi}$ *Corresponding author; Email:* khirod555@gmail.com drinking water, eating fish, sea food and using cosmetics or any other manmade things in day-to-day life. Although some metals essentially required for various metabolic activity in body. However higher exposure to these heavy metal leads to various negative health impacts. Metals like lead is highly toxic for human especially to children, arsenic associated with respiratory disorder, lung infection and cardio vascular disorder. Chromium is the potent carcinogen and nickel is very dangerous as it causes lungs cancer, allergies, various kidney, and heart related disease. Exposure to cadmium also causes various diseases in human beings (Loh *et al.*, 2016; Coetzee *et al.*, 2020; Genchi *et al.*, 2020). However, in biosphere the amount heavymetal deposition increasesrapidly.

Though characterization of Physio-chemical parameters like precipitation, reverse osmosis, heat treatment, ion exchange, soil washing, solidification and chemical leaching are actively involved in soil remediation, it changes soil properties especially pH, nutrient profile, which in turn reduces soil fertility and it also have small-scale applications. (Nedjimi and Daoud 2009)

Phytoremediation is use of plant to detoxify pollutant from soil and waterbodies. The process of phytoremediation is executed based on five different mechanisms. It depends on hyperaccumulating potential of plant (phytoextraction) and rhizospheric microorganism to stabilize metal (phytostabilization), transfer the pollutant from toxic to nontoxic or less toxic volatile form (phytovolatilization) and degradation of pollutant inside plant body (phytodegradation) (Ghosh and Singh, 2005). As compared to other physical techniques, phytoremediation is well efficient, eco-friendly, low-cost, large-scale application, easy to dispose and most importantly improves soil fertility by releasing organics matters (Saier and Trevors, 2010). Research has already been carried out to enhance phytoremediation process which includes the use of aids like increase soil amendment with biochar (Sugawara *et al.*, 2022), using EDTA (Kamal *et al.*, 2023), different biotechnological approach to produce genetically engineered plant (Bhuiyan *et al.*, 2011). These aids enhance phytoremediation by providing availability of heavy metal, support plant growth and in certain circumstances reducing the toxicity of pollutant. The main objectives of the review is to understand the efficacy of some known hyperaccumulator for its integrated approach in phytoremediation and biomass utilisation through different process. Some plant species such as *Pteris vittata,* different *Brassica* species (*B. juncea, B. napus, B. campestris),* different species of *Asteraceae (Tagetes erecta*, *T.patula, Helianthus annuus*, *Helianthus petiolaris) and Ricinus communis, Jatropha curcas and Cannabis sativa* are successfully involved in extraction of heavy metal like arsenic (As), cadmium (Cd), lead (Pb), nickel (Ni), zinc (Zn), iron (Fe), manganese (Mn) from polluted soil. Following the accumulation of the heavy metals, proper utilization of their plant biomass occurs in the field of biofumigation, biofortification and for bioenergy production (Table 1). This is because the efficiency of phytoremediation not only depend upon by choosing right hyperaccumulators but also on selective utilization of plant biomass to generate bio-product which are used in further processes.

1.1. Criteria of different plant species used in phytoremediation

The plant used in phytoremediation must deal with wide range of pollutants rather than focusing on specific one. Plant should exhibit higher biomass production in above ground part and also possess extensive root system. Moreover, it must be hyperaccumulator, capable of tolerating heavy metal toxicity, easy to cultivate, and show resistance to herbivory (Adesodun *et al.*, 2010).

1.2. Plant species used in phytoremediation

There are approximately 400 plant species belonging to 45 families are reported for hyperaccumulating metal or metalloids (Ghosh and Singh, 2005). Most commonly, hyperaccumulator species belong to families such as Brassicacece, Asteraceae, Euphorbiaceae, Lamiaceae, and Scrophulariceae. Among these, most of the hyperaccumulator are member of family Brassicaceae. The use of the first transgenic plant in phytoremediation of selenium-polluted soil was successfully confirmed by Bauelos *et al.* (2005) and suggested *B. juncea* with overexpressed glutathione synthetase (GS) exhibit significant tolerance for selenium and had more biomass than wild type plants. Similar results were drawn *B. juncea* as a potent species showing highest tolerance to Cd and Pb due to presence of BjGSII (*Brassica junceag* lutathione synthetase II) and BjPCS1 (*Brassica juncea* phytochelatin synthase 1) induced by over expressed AtATM3 gene. Gurajala *et al.* (2019) investigated the impact of bi-metal (Pb and Cd) contaminated soil with various genotypes of *B. juncea* and confirmed genotypes IM-13, IM-25, IM-65 were more effective for removing Cd followed by IM-24, IM-79, IM-32. Kamal *et al.* (2023) investigated dependent effect of EDTA (Ethylenediaminetetraacetic acid) on *B. juncea* seedlings and concluded highest plant biomass found in 2mM per kg EDTA. Beyond this, both the photosynthetic activity and plant biomass decreased. It was also reported that exogenous supply of EDTA. On contrary, Afshan *et al.* (2015) opined application of citric acid enhanced plant growth, biomass, chlorophyll, significant

Table 1:

Various methods of utilisation of phytotremidiated plant biomass in different families.

Sl No	Plant Family	Biomass utilization process	Outcomes	Reference
1.	Brassicaceae	Biofumigation	Highest antifungal potency exhibited in seed meal inhibiting 61.5% of fungal growth, followed by seed powder, flowering stage, and vegetative stages of fresh plant part in *Brassica juncea.*	Abdallah et al., 2020
2.	Brassicaceae	Biofumigation	White cabbage showed a positive relationship between heavymetal accumulation and the production of bioactive glucosinolates (GLS) production.	Kusznierewicz et al., 2012
4.	Brassicaceae	Biofortification	*Brassica rapa* ESB1 mutant exhibited biofortification for Fe and Cu.	Calvo et al., 2023
5.	Asteraceae	Bioenergy	Oil and bioethanol yield from of *Helianthus annus* is consistent in both agricultural and industrial soil polluted with Zn and Cd.	Paulo et al., 2023
6.	Pteridaceae	Biochar	Phyto remediated biomass of *P. vittata* used as biochar and supply with $FeCl_3$ enhanced the arsenic adsorption by biochar.	Sugawara et al., 2022
7.	Asteraceae	*Biochar*	Biochar obtained from pyrolyzed biomass of *H. annuus* were reutilized as fertilizer.	Zhou et al., 2020
8.	Pteridaceae	Bioenergy	Coupling with ethanol extraction and anaerobic digestion reduced 98% arsenic concentration in *P. vittata* biomass.	Silva et al., 2019
9.	Cannbaceae	Biofortification	*C. sativa*had potential *to* accumulate Selenium in leaves and seed which in turn show biofortifying potential.	Stonehouse et al., 2020

rise in antioxidant enzyme with notable increase in chromium uptake and minimized Cr induced stress in *B. napus.* By analysing bioconcentration factor and morpho-physiological, biochemical analysis, Ali *et al.* (2022) confirmed phytoextraction potential of *Brassica* species in the order of *B. juncea*> *B. napus* > *B. campestris* > *B. rapa* on contaminated soils. Recently Bortoloti and Baron (2022) concluded phytoremediation by *Brassica* species was the most promising approach but it needs further studies to assist utilization of biomass and tolerance.

Biswal *et al.* (2021) investigated efficacy of *Tagetes erecta* and *Tagetes patula* on removal of Cd and Ni from polluted site and observed *T. erecta* and *T. patula* are quite effective at removing Cd and Ni, respectively and as comparison to *T. patula*, *T. erecta* had higher biomass and it more efficiently accumulated heavymetal from contaminated soil. Similarly, Madanan *et al.* (2021) confirmed *T. erecta* showned BCF >1 for Cd and Zn but <1 for Pb which clarify *Tagetes erecta* L. was an efficient hyperaccumulator of Zn and Cd and excluder of Pb. Francis (2018) screened the Phyto-remediating potential of *Helianthus annuus* and observed that the metals are accumulated in various part of plant in the decreasing order of Ni > Pb > Cr > Cd in the plant part. In contrast, Aybar *et al.* (2023) observed *Helianthus annuus* was the good phytoextractor of zinc and lead and stabilized copper in soil found in vicinity of mining area. Sharma and Mathur (2023) suggested *H. annuus* effectively extracted zinc as compare to *T. erecta* and utilized as successful phytoextractor of zinc from polluted soil.

Jatropha curcas effectively accumulated considerable amount of Fe and also extract Pb, Zn, Cu, Cr and Ni with little amount of As, Hg, Sn (Mateos *et al.*, 2019). Kristanti *et al.* (2023) confirmed, *J. curcas* was abled to extract 88.5% of aluminium and showed bioconcentration factor up to 5.62

which indicates potent hyper-accumulator of aluminium. Jain and Tembhurkar (2023) evaluated the potential of remediation and energy yield of *J. curcas, Millettia pinnata* & *H. annuus* in fly ash contaminated soil and observed that *J. curcas* was much efficient phytoremediator. However, *H. annuus* accumulated higher heavymetal but it no longer survived in such condition.

In *Ricinus communis,* the order of metal accumulation was observed as Fe > Zn > Mn >Pb >Cd, which is negatively co-related with concentration of protein and chlorophyll content (Boda *et al.*, 2017). *Ricinus communis* trans located lead in aerial part of plant in the order of shoot > root > leaf and used as an indicator of Pb in contaminated soil (Roychowdhury *et al.*, 2019). Similarly Khan *et al.* (2019), *R. communis* stored considerable amount of heavymetal in aerial parts which reduced heavy metal content of soil.

Pteris vittata L, hadability to withstand at very high concentration of arsenic and stored in its frond. Gaggero *et al.* (2020) observed, *P. vittata* efficiently removed arsenic, while *B. Juncea* showed higher translocation and bioaccumulation for Cadmium and *H. annuus* successfully extracted zinc and cadmium whether as no such result was found in *Zea mays*. These finding also provides the differential accumulation potential of different species for extraction of heavymetal from contaminated sites and recommend the use of plant species in accordance to the target. Kohda *et al.* (2022) observed *Pteris vittata* extracted 2.82 kg arsenic per hector in sub-arctic area which was quite efficient for removal of heavymetal in phytoremediation under such condition. *Pteris vittata* significantly accumulated As and Pb in co-planting but after addition of chitosan, the uptake of Cd and Pb by *Pteris vittata* and *Ricinus communis* increased significantly. However, accumulation of As by *Pteris vittate* was reduced (Yang *et al.*, 2017). Wan *et al.* (2021), explored intercropping system with *Pteris vittata* to enhance the accumulation of arsenic while simultaneously decreasing the concentration of Arsenic in another crop. This study givesnew direction for improving phytoremediation of Arsenic polluted soil with *Pteris vittate* by intercropping methods.

Cannabis sativa accumulates Cu, Cd, and Ni in the leaves due to presence of the stress tolerant genes PLD and GSR in response to heavymetal (Ahmad *et al.*, 2016). Picchi *et al.* (2022) put forward that *C. sativa* showed higher BCF and lower TF for Arsenic as compare to *B. juncea*. However, exogenous supply of phosphate had no role in accumulation of heavymetal but it only increases plant physiological functions. Testa *et al.* (2023) observed that in comparison to Cd and Pb, Ni significantly reduced the biomass of *Cannabis sativa* and further 75 cultivar had more Cd and Pb tolerance *C. Sativa* completed its life cycle until seed bearing phase even in heavily polluted soil which provides new insight for the creation of green energy. The current study provides new perspectives for effectively choosing hyperaccumulator species for long-term phyto-management of a substantially contaminated site using a combined phytoremediation-bioenergy approach for clean-up soil and sustainable development.

2. Integrated approach for utilisation of plant Biomass

2.1. Biofumigation

Generally, the families of order Capparales (Brassicaceae, Capparaceae, Moringaceae) contains more glucosinolates than other families (Euphorbiaceae, Salvodaraceae (Fenwick *et al.*, 1983). Glucosinolates (GSLs) are secondary compounds present in the order Capparales. The breakdown products of GSLs offer a broad range of defence against pathogens (bacterial, fungal, nematodes) and herbivores. Additionally, GSLs play a crucial role in determining the taste and smell of cruciferous vegetables, providing various health benefits. The amount of glucosinolates (in vacuoles) increases when the plant is under attack from a pathogen or experiences stress, and as it comes in contact with myrosinase (in the cytoplasm), it hydrolyzes the thioglucoside bond and releases biologically active isothiocyanates (ITCs), thiocyanates, nitriles, etc. (Andernach *et al.*, 2023). Wu *et al.* (2011) fund the notable decrease in nematode motility upon exposure to isothiocyanates (ITCs) from *Brassica* plants. This observation is further supported by Fourie *et al.* (2016), who highlighted the successful utilization of species such as *B. oleracea*, *B. rapa*, *B. napus*, *B. juncea*, *B. campestris*, *B. nigra*, *E. sativa*, *R. sativus*, etc., for biofumigation. *Brassica* as a cover crop hada number of benefits since it combats soil pathogens, nematode, weeds (Nyczepir *et al.*, 2009) and reduced soil borne disease, as well as enhanc soil fertility and reduce soil erosion (Nyczepir *et al.*, 2009).

According to Kusznierewicz *et al.* (2012), heavy metal accumulation and generation of bioactive glucosinolates (GLS) are positively correlated in cabbage. However, rise in glucosinolates level occurred in a dose-dependent manner which proves excellent bio-fumigation potential of white cabbage. Jakovljeviæ *et al.* (2013) examined effect of Cadmium toxicity with respect to change in concentration of glucosinolates and sulphurcontent and found heavy metals did not significantly alter plant biomass or cause any harmful symptoms. However, the root showed higher Cd accumulation as translocation from root to shoot become saturated. Contrarily, Durenne *et al.* (2018) found increasing Cd dosages result in decrease amount of glucosinolates in

B. napus roots, shoots while increasing the amount of sulphur in other plant parts. Abdallah *et al.* (2020) confirmed biofumigant effect of *B. juncea* and found seed meal (without fat) was more potent by inhibiting 61.5% fungal growth followed by seed powder and flowering stage as compared to vegetative stages. Biofumigation is not only limited to Brassicaceae but also in different families other than it and produce cyanogenic compound which is pathogen repellent in nature. Marigold belongs to Asteraceae, produce α-terthienyl which activated upon ultraviolet range, produce reactive oxygen species which was phytotoxic in nature and also had broad effect against pathogen. Several allelopathic compound like diethynyl, dithioacetylene also possess biocidal property (Dutta *et al.*, 2019). Further study must focus to evaluate the effect of heavymetal concentration and glucosinolates production in plant, the mechanism of activity and the metabolic pathway concerned with glucosinolate production and role of glucosinolate in reduction of phyto-toxicity arise due to heavymetal.

2.2. *Biofortification:*

High population growth, climate change and deficiency of nutrious food are the major reason of malnutrition in developing countries. Zn and Fe deficiency in human is common in the areas where soil is poor Zn and Fe or have antinutrient which inhibit Fe and Zn absorption (Cakmak, 2008). Several studies of plant genetic engineering along withcrop breeding performed to enhance nutrient like Feins food crops by increased expression of metal binding protein, chelating agent, amino acid (Kumar *et al.*, 2019), increase translocation of Fe and Zn from root to aerialpart and reducing the effect of anti-nutrient phytate (Palmgren *et al.,* 2008). There was great variation among genotype *Brassica* species but it considered as good source of nutrient (Fe and Zn) and substantial amount present in young part of plant during their growth (Gioia *et al.*, 2017). Vegetables belonging to family Brassicaceae have capacity to accumulate selenium (Se) which is an essential micro nutrient and produce seleno compound with numerous health benefits. *B. juncea* successfully extracted selenium from coal mining waste and also accumulates minute amounts of As, Cd, Pb, and Cu (Monei *et al.*, 2021). Although *Brassica rapa* ESB1 mutant did not show biofortification for Selenium however, it minimizes translocation of Cd in the leaves and increase Fe and Cu uptake (Calvo *et al.*, 2023). *Cannabis sativa* also showed biofortifying potential in Se contained agricultural land and accumulated Se in leaves and seed (Stonehouse *et al.*, 2020). Moreover, selective study needs to focus on successful biofortification of essential nutrient in crop from contaminated area.

2.3. *Bioenergy*

The efficacy of integrated phyto-remediation with bioenergy production assist environment by removing pollutant, better soil quality as well as a viable alternative for sustainable energy source. Bioenergy is a type of renewable energy, generated from biomass of living organism especially plant and generated biomass from phytoremediation process act as indirect source of solar energy. So, it can be used as energy production in different process like Combustion, gasification, and pyrolysis methods. Several plant species have the potential to execute phytoremediation with production of bioethanol, biodiesel, biogas, biochar, charcoal etc. Park *et al.* (2012) successfully extracted heavymetal from the soil and the oil extracted from *B. napus* biomass were used as biodiesel as it contains heavymetal at standard permissible level. Huang *et al.* (2018) observed pyrolyzed temperature increase from 550 °C, there was sharp decrease in heavymetal concentration in plant biomass of *Jatropha curcas* and it was safe for use. Hunce *et al.* (2019) found the seeds of *H. annuus* produced highest biogas in anaerobic digestion which further satisfy sustainable green energy from hazardous Phyto-biomass.

Cannabis sativa was a potent hyper accumulator of heavymetal in polluted site with characteristic high biomass which utilized for production of biogas, bioethanol, biodiesel, bio methanol (Kumar *et al.*, 2017). Todde *et al.* (2022) opined on *C. sativa*that anaerobic digestion and incineration of that biomass are used for most sustainable production of energy by reducing the production of CO_2 into the atmosphere. By combining ethanol extraction and anaerobic digestion, Silva *et al.* (2019) created a unique approach for decreasing arsenic in *Pteris vittata* biomass by 98% and remaining Arsenic in *Pteris vittata* biomass consider as safe material based on standard toxicity limit. The combination of integrated phyto-remediation with bioenergy production offers a novel perspective on the sustainable production of energy, presenting an alternative method for utilizing plant biomass.

2.4. *Biochar*

Biochar is the carbonized product produced from thermal decomposition of plant biomass anaerobically and used as soil conditioner, carbon dioxide sequestration, and in energy production. Nevertheless, it is crucial to regulate the quality of biochar, especially when it produced from waste source (Lehmann and Joseph, 2015). Zhou *et al.* (2020) provided an innovative approach to reduce the bio hazardous phytobiomass of *H. annuus.* From pyrolyzed biomass, metals were separated by acid extraction followed by alkaline precipitation method and the biochar obtained from this were reutilized as fertilizer. Lee *et al.* (2021) reconfirmed same result in *H. annuus* where production of hydrochar resulted in heavy metal content within a safe range. Sugawara *et al.* (2022) observed phyto-remediated biomass of *Pteris vittate* when transformed into biochar and treated with $FeCl_3$, indicate rapid raised in arsenic adsorption. Although biochar production required high temperature however, more study would reveal the sustainable production of biochar from phytoextracted biomass and proper utilization in soil amendment.

3. Summary and future perspectives

Schematic diagram (Figure 1) reflects the heavy metal sources and their deposition in soil. The concentrated heavy-metals are minimized by using green technology called phytoremediation. In this the plant extract heavy metal from soil (phytoextraction), converts to less toxic form (phytovolatilization), and stabilize heavymetal in soil (phytostabilization). Later, the biomass was used to produce biochar, which assisted in phytoremediation to adsorb heavy metals. Furthermore, it also utilized to produce bioenergy (bioethanol, biofuel). Biomass also utilised as biocides and enrich nutrient in crop by bio fumigation and biofortification respectively. Further study must be focused on metabolic pathway, enzymes, gene expression, fate of heavymetal inside plant tissue, which provide tolerance to plant species and efficiently extract heavymetal from polluted sites. Further research is required to comprehend the production of glucosinolates or cyanogenic glycosides induced by heavy metals. This can lead to the development of natural biocides with fewer environmental side effects.

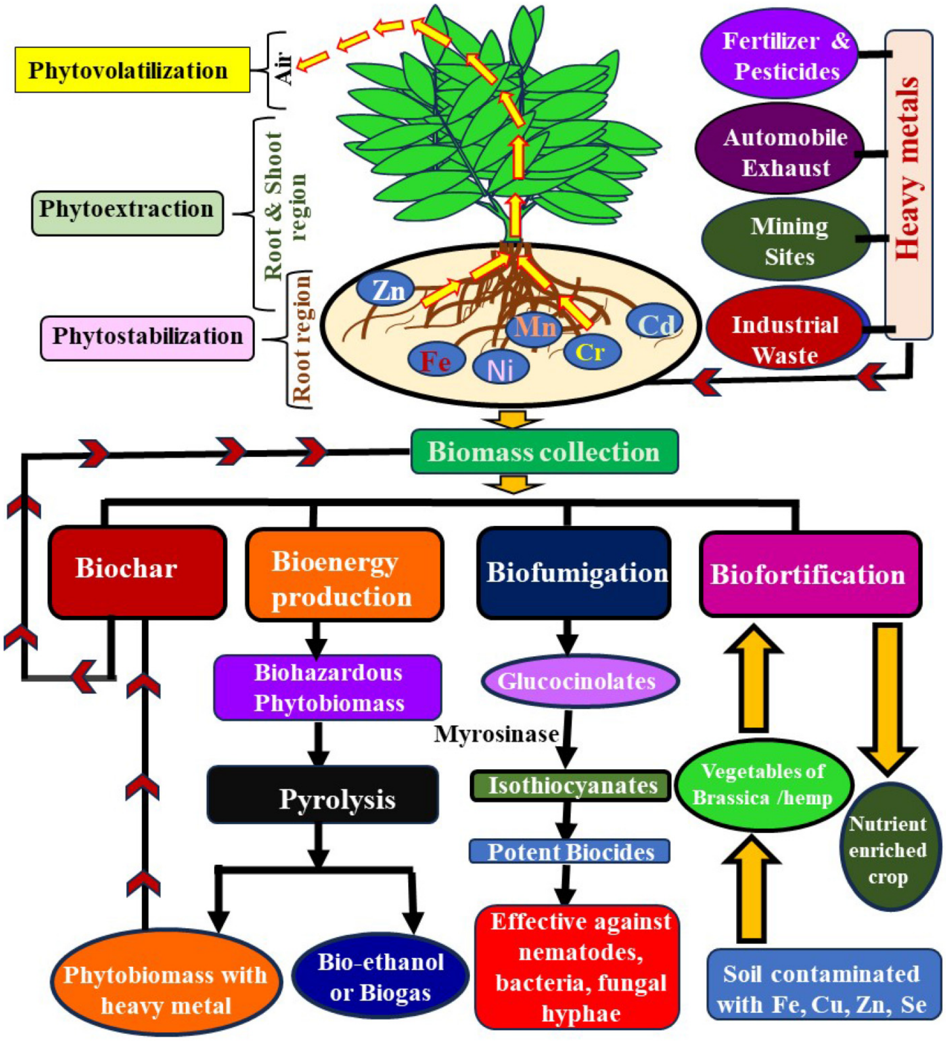

Figure 1: Schematic diagram showing integrated approaches towards efficient phytoremediationby using green technology. It summarises various sources of heavy metals, their deposition in soil, the ways to minimize though phytoextraction, phytovolatilization and phytostabilization. In the next step, the biomass was used to produce biocharto adsorb heavy metals, to produce bioenergy (bioethanol, biofuel), biocides for better enrich nutrient in crop by bio fumigation and biofortification.

4. Conclusion

In the current scenario, the problem associated with heavymetal is a persistent issue. Phytoremediation is emerging solar energy driven technique which utilizes the plants for removal of heavy metal (inorganic and organic form) without disturbing the physiological characteristic of soil. Phytoremediation of metals may be enhanced by using aids like soil amendment with biochar, EDTA, citric acid, rhizospheric association between endophytic bacteria, other biotechnological approaches to produce genetically engineered plant. Efficient phytoremediation of heavymetals requires the proper utilization of plant biomass using hyperaccumulator. Future research in this area would provide new insight for sustainable development of ecosystemas well as the toxic heavy metal can successfully be extracted and efficiently employed for generation of bioproduct using the integrated approaches.

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