Original Research Article

Enrichment of Cd, Cr and Pb and Biological Detoxification Strategies in Sewage-Irrigated Soils

Abstract

Aim: Long-term use of sewage-sludge and improper management of agricultural lands can lead to an elevated heavy metal concentration in the soil resulting in barren soil. The present investigation was carried out to study the influence of different soil characteristics on the enrichment of Cd, Cr and Pb and their detoxification strategies in sewage irrigated soil profiles of Prayagraj region, India.

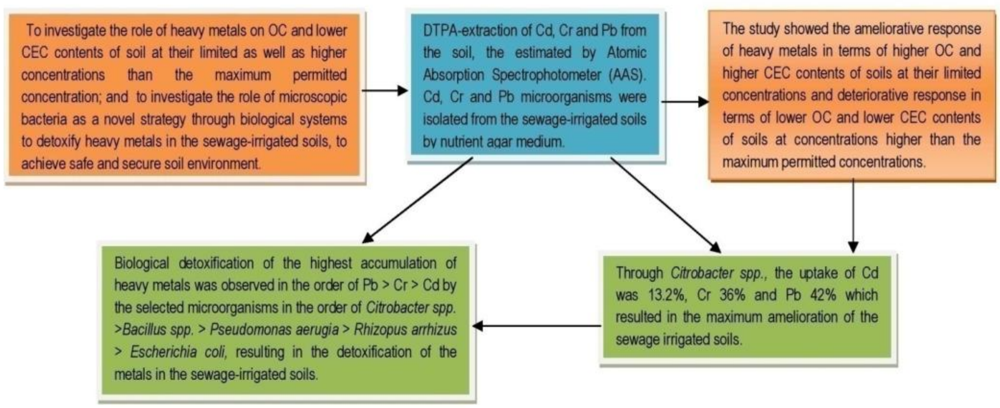
Methodology: Di-acid mixture method used for soil analysis. The clean filtrate was used for the estimation of the heavy metals (Cd, Cr and Pb) by Atomic Absorption Spectrophotometer (AAS) (AAnalyst600, Perkin Elmer Inc., MA, USA). Isolation and identification of Cd, Cr and Pb Detoxifying microorganisms were isolated from the sewage-irrigated soils by nutrient agar medium.

Results: It was observed that surface soils contained higher amount of DTPA- extractable heavy metals (Cd 3.00-3.20 mg kg^{-1},Cr 5.20-6.40 mg kg^{-1} and Pb 6.80-7.20 mg kg^{-1}) and these amounts along with CEC (26.10 Cmol (p^+) kg^{-1}) and organic carbon (OC) Content (0.58%) decreased with the depth of soil profiles. Among the tested microorganisms, the highest accumulation of heavy metals in the sewage irrigated soils was observed in *Citrobacter spp.* to the extent of Cd 13.2%, Cr 36%, and Pb 42%. However, the lowest accumulation was observed in *Escherichia coli* to the extent of Cd 1.2%, Cr 8.4%, and Pb 10.2%.

Conclusion: The horizons A of the studied soil profiles contain almost 12% more Cd, 9.11% more Cr, and 52% more Pb as compared to the contents of horizons B. Biological detoxification system metal uptake is largely influenced by the availability of metals. The highest accumulation of heavy metals was observed in the order of Pb > Cr > Cd by the selected microorganisms in the order of *Citrobacter spp.* >*Bacillus spp.* > *Pseudomonas aerugia* > *Rhizopus arrhizus* > *Escherichia coli,* resulting in the detoxification of the metals in the sewage-irrigated soils.

Keywords: Biological importance, bioremediation, enrichment, heavy metals, sewage sludge, soil profile

Graphical Abstract



Introduction

The majority of heavy metals are created by pollution, and their presence has a negative impact on nutrition, evolution, and the environment. Heavy metal contamination poses numerous problems, including soil degradation and concerns about the safety and quality of food (Christophoridis et al. 2020; Zaheer et al. 2022). Plants are among the many living things that heavy metals affect. Compared to other metals, they inhibit plant growth and development even at low concentrations of heavy metals. Other metals or elements present in excess do not harm the plant's tissues or cells; in fact, their buildup may promote the plant's growth. The metals which are lethal or harmful for plants include: lead (Pb), cadmium (Cd), cobalt (Co), iron (Fe), silver (Ag), platinum (Pt), nickel (Ni), chromium (Cr), copper (Cu), and zinc (Zn) (Pescatore et al. 2022).Both natural and human-made processes are the sources of heavy metals in the surface environment. Parent rocks and metallic minerals are examples of natural sources. According to Navratil and Minaril (2005, Odika et al., 2020), anthropogenic causes include energy production (power plants, leaded petrol, etc.), metallurgy (mine, smelting), agriculture (fertilisers, pesticides, etc.), and sewage

Comment [WU1]: Please check these citations disposal. According to Elgallal et al. (2016), the prolonged application of partially or completely untreated wastewater may result in the accumulation of heavy metals in the soil. Several pollutants are carried by sewage and/or irrigation system runoff, drainage water, atmospheric deposition, and traffic-related emissions, which enrich urban wastewater with heavy metals (Saha et al., 2015; Zia et al., 2016; Woldetsadik et al., 2017).

In addition to meeting the diet's daily caloric needs, vegetables also include specific photochemicals with antioxidant properties, such as vitamins, carotenoids, and polyphenols. The beneficial metabolic role of these phytochemicals in shielding people from oxidative stress or free radicals has recently attracted more attention. One of the key endogenous phytochemicals for these species' defence mechanisms is glutathione (GSH). *Brassica* species are farmed all over the world, particularly as dietary vegetables, but little to no attention has been given to the biochemical mechanisms of metal translocation in plants and the relationship between this and the capacity of plants to accumulate excessive amounts of metal in sewage-irrigated soils. With relatively little knowledge of the biochemical reasons of phytoremediation, the authors previously described the potential for phytoremediation of common sunflower in sewage-irrigated Indo-Gangetic alluvial soils (Mani et al., 2012). The primary non-ferrous industrial units, Raymonds Synthetic Ltd., Hindustan Cable Ltd., Deys Medicals, Sangam Structural's, Swadeshi Cotton Mills, Bharat Rocklike Plastics, Petroleum Corporation Ltd., Indian Telephone Industry, and Baidyanath, contaminate the vegetable production sites in the urban and sub-urban areas surrounding Prayagraj city with sewage-irrigation.

Numerous factors, including pH, EC, clay content, organic matter content, and the physical and mechanical properties of the soil, affect the absorption and accumulation of metals in plants. According to El-Zabalawy et al. (2015), plants absorb heavy metals from soil through a variety of processes, including ionic exchange, redox reactions, and absorption. It increases soil fertility and soil organic carbon, which aids in raising agricultural yields. Heavy metals like chromium (Cr), cadmium (Cd), and lead (Pb) accumulate significantly when sewage water is used for irrigation over an extended period of time. Zn, Cu, Ni, and Mercury (Hg) in soil that enters the human body through the food chain (Dotaniya et al., 2017). Lead is known to be hazardous to plants, animals, and microbes when present in the environment. Since Pb^{2+} is not biodegradable, it persists in soil for a long time after contamination, having a negative impact on biological processes (Pehlivan et al., 2009). According to Cho-Ruk et al. (2006), pb exposure causes substantial risks to human health, including brain damage and retardation. It inhibits a number of

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fundamentally important physiological and biochemical processes (Kosobrukhov et al., 2004), leading to overt poisoning symptoms and ultimately reducing vegetative and reproductive growth (Rossato et al., 2012).

Sites that had low amounts of heavy metals initially but were contaminated by human activities are the candidates for soil detoxification. Utilisation of microorganisms for metal detoxification via valence transformation, extracellular chemical precipitation, or volatilization (certain bacteria are capable of reducing a variety of metals enzymatically in metabolic processes unrelated to metal absorption) (Lone et al., 2008). Metalloproteinases like phytochelatins, glutathione, and metallothioneins regulate and detoxify heavy metals in living things. The metal regulating mechanism may be altered by metal concentrations over the limit, which could lead to metal accumulation (Kumar et al., 2017; Maurya and Malik, 2018). Because they act as mutagenic and carcinogenic chemicals, heavy metals constitute a serious threat to the environment and to living things, unlike organic pollutants, which can biodegrade (Wu et al., 2018).

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With the aforementioned information in mind, the current study was carried out to evaluate the depth-wise distribution, mobility, and detoxification approach of Cd, Cr, and Pb in sewage-irrigated surface and sub-surface soil profiles. The main goals were to: (1) investigate the impact of heavy metals on OC and lower CEC contents of soil at their limited as well as higher concentrations than the maximum permitted concentration; and (2) investigate the impact of microscopic bacteria as a novel biological strategy to detoxify heavy metals in the sewage-irrigated soils, to achieve a safe and secure soil environment.

Methods and Materials

The experimental location is located in northern India at 24°58ʹ N latitude and 80°56 E longitude, facing slopes of comparable inclination at heights between 170 and 85 m above sea level, and is bounded by the Himalayas to the south and the Arabian Sea to the north. A soil sample was taken from Prayagraj, India, and it came from the sewage-sludge-irrigated Indo-Gangetic alluvial soils of the Sheila Dhar Institute of soil science farm located on the confluence of the Ganga and Yamuna alluvial deposit. The properties of the soil were: pH 7.7±0.25, EC 0.26±0.02 dSm^{-1}, organic carbon (OC) content 0.58±0.03 %, total N 0.07±0.03 %, total P0.04±0.03%, CEC 18.4±2.18 C mol $(P^{+})kg^{-1}$, Cd 3.00-3.20 mg kg^{-1},Cr 5.20-6.40 mg

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$kg^{-1}$and Pb 6.80-7.20 mg kg^{-1}. The texture comprised of sand (>0.2mm) 55.0±5.2 %, silt (0.002-0.2mm) 21.0±4.5% and clay (<0.002mm) 24.0±4.5 %.

DTPA extraction of Cd, Cr, and Pb from soil and soil sampling: Each sampling unit had numerous locations where soil samples were taken in a zigzag manner, leaving a 2 m-wide area along the field edges. The Pipette method was used to separate silt and clay, while decantation was used for fine sand. 2 g of soil were combined with 5 ml of HNO_3 (16 M, 71%) and 5 ml of HClO4 (11 M, 71%) to determine the total Cd, Cr, and Pb content. The composite was dried out using heat. The volume was heated distilled water to a volume of 50 ml. The Atomic Absorption Spectrophotometer (AAS) (AAnalyst600, Perkin Elmer Inc., MA, USA) was used to estimate the heavy metals (Cd, Cr, and Pb) in the clean filtrate.

Soil Physico-chemical analysis: An electrical digital pH metre was used to measure the pH of the soil using a 1:2.5 soil-to-water ratio. For the preparation of the chromic acid digestion method, neutral 1 N ammonium acetate solution, the cation exchange capacity (CEC), the total nitrogen by digestion mixture (containing sulphuric acid, selenium dioxide, and salicylic acid), and the total phosphorus by hot plate digestion with HNO_3(16M, 71%) and extraction by standard ammonium molybdate solution, double distilled water was used.

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Isolation and identification of Cd, Cr and Pb Detoxifying Microorganisms: Microbial strains were isolated from the collected soil samples by serial dilution technique. Selective isolation of bacterial species was performed by spreading the samples on their individual media (Gislin et al., 2018). Individual distinct colonies were further undergone repeated sub-culturing. Selected colonies were grown on nutrient agar media (Prayagraj, India). Identification of microbes was based on the morphological, cultural and biochemical characteristics for the activities of motility, oxidase, catalase, VP test, MR test, starch and gelatin hydrolysis, indole production and citrate utilization according to Bergey's manual of systemic bacteriology (Bergey and Holt, 1989).Cd, Cr and Pb microorganisms were isolated from the sewage-irrigated soils by nutrient agar medium (5.0 g peptone, 1.5 g beef extract, 1.5 g yeast extract, 500 g sewage irrigated soils, 500 ml deionized water and 15 g agar) amended with 10 mg l^{-1} Cd, Cr and Pb (as $CdCO_3$, $CrCl_3$ and $PbCO_3$).

Statistical Analysis: The experimental results were expressed as mean ± standard error of mean (SEM) of three replicates. Graphpad Prism version 8, software was using for drawing Figures.

Results and Discussion

The pH of the two profiles, Naini and Jhunsi, was high (7.7 pH) and generally rose with depth. The heavy metal levels of the two profiles are highest in the sewage-irrigated brown soils of Naini profiles and lowest in the sandy clay soils of Jhunsi profiles (Table 1). Independent of the soil type, the distribution pattern of the heavy metals in the soil profile is often uniform. Lead, cadmium, and chromium content, however, is higher at the surface strata and dramatically declines with depth (Fig. 1).

Enrichment of DTPA-extractable Cd, Cr and Pb in sewage-irrigated soil profiles: There is a significant relative enrichment of heavy metals in these soils because of the rapid weathering of carbonate minerals (Nowacket al., 2001). The Naini profile, receiving comparatively higher sewage-sludge than the other two profiles, contains elevated OC concentrations at the 15-cm depth correspond to a darker color at this depth in the profile. The profile of Jhunsi is most likely characterized by sedimentation of eroded topsoil in the depression, which is moderately polluted both the two profile (Table 1). The higher retention of in the upper horizon in comparison to the lower horizon of soils (Fig. 1). DTPA-extractable Cd of soil profiles presented in their content in the surface horizon ranged from 3.0-3.20 mg kg^{-1}, whereas, in the lowermost horizon Cd concentration varied from 0.10-0.20 mg kg^{-1}, respectively (Table 1). Moreover, it refers to the concentration of cadmium and chromium from sewage-sludge and lead from atmospheric deposition (Sipos, 2004).Higher retention of these heavy metals was observed in the upper horizon in comparison to the lower horizon which was closely correlated with organic matter (OM) and CEC distribution in the profiles.

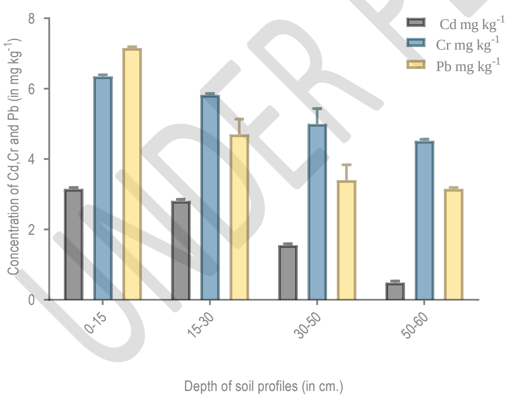

Fig. 1: Depth-wise enrichment of DTPA-extractable heavy metals in Prayagraj region

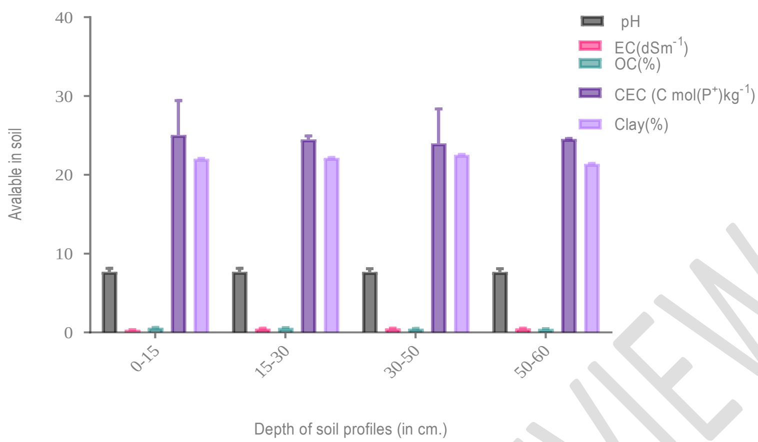

Fig. 2: Depth-wise distribution of physicochemical properties of soil profiles in Prayagraj region

Table 1: Distribution of DTPA-extractable heavy metals in sewage-irrigated soil profiles of Prayagraj region

Site	Depth (cm)	pH	EC (dSm^{-1})	OC (%)	CEC Cmol(P$^+$)kg^{-1}	Clay (%)	Cd mg kg^{-1}	Cr mg kg^{-1}	Pb mg kg^{-1}
Naini	0-15	7.8±0.34	0.32±0.06	0.6±0.04	24±2.64	23±3.60	3.2±0.11	6.4±0.04	7.1±0.04
	15-30	7.8±0.2	0.43±0.06	0.58±0.06	24±4.35	22±2.64	2.8±0.08	5.8±0.08	5.2±0.02
	30-50	7.7±0.2	0.54±0.04	0.45±0.01	25±3.60	22.1±4.64	1.62±0.07	5.2±0.09	3.4±0.08
	50-60	7.7±0.34	0.56±0.02	0.46±0.03	26.1±3.53	21±4.58	0.54±0.06	4.9±0.03	3.2±0.05

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Jhunsi	0-15	7.6±0.36	0.33±0.04	0.58±0.05	26.1±4.32	21.2±5.75	3.1±0.08	6.3±0.03	7.2±0.08
	15-30	7.6±0.36	0.44±0.03	0.56±0.04	25±6.24	22.3±3.51	2.83±0.12	5.85±0.08	6.1±0.03
	30-50	7.5±0.4	0.46±0.08	0.48±0.06	23±3.60	23±2.64	1.48±0.05	4.78±0.05	3.4±0.04
	50-60	7.5±0.55	0.54±0.06	0.44±0.05	23±3.0	21.8±2.25	0.45±0.07	4.2±0.06	3.1±0.09
Mean	0-15	7.7±0.3	0.32±0.06	0.59±0.08	25.05±3.63	22.05±5.55	3.15±0.04	6.35±0.04	7.15±0.08
	15-30	7.7±0.26	0.43±0.08	0.57±0.04	24.5±4.92	22.15±3.39	2.81±0.06	5.82±0.06	4.7±0.04
	30-50	7.6±0.36	0.5±0.04	0.46±0.02	24±6.24	22.55±6.08	1.55±0.02	5±0.07	3.4±0.03
	50-60	7.6±0.26	0.55±0.02	0.45±0.03	24.55±3.30	21.4±1.57	0.49±0.05	4.55±0.02	3.15±0.05

Distribution of physicochemical properties and Cd, Cr and Pb of soil profiles: When compared to the contents of horizons B, the horizons A of the examined soil profiles include nearly 12% more Cd, 9.11% more Cr, and 52% more Pb. A higher concentration of air pollutants was the reason of the higher lead buildup. According to Goel et al. (2015), car exhausts may be to blame for the rising trend in Pb levels. Due to the release of industrial waste from dye factories, tanneries, and batteries, the Cr and Cd concentration increased. The first table availability of Cd, Cr, and Pb in soils was altered by variations in depth of profiles from 0 to 60 cm, EC from 0.32 to 0.55 dSm^{-1}, CEC from 25.05 to 24.55 Cmol kg^{-1} of soil, OC concentration from 0.45 to 0.59%, and pH alterations from 7.6 to 7.7. The study demonstrated how heavy metals responded favourably in terms of higher OC and higher CEC contents of soils at their permissible concentrations and negatively in terms of lower OC and lower CEC contents of soils at concentrations more than the maximum authorised values. (Fig.2).

Detoxification of Cd, Cr and Pb in sewage irrigated soils: Microscopic bacteria have developed metal resistance tactics to combat the debilitating effects of harmful severe metals. Microorganism communities have adopted the following methods for metal detoxification: (1) metal exclusion by porous barriers, (2) metal transport from the cell, (3) intracellular sequestration of the metal by supermolecule binding, (4) living thing sequestration, (5) catalyst detoxification of metal to a less harmful kind, and (6) lowering the sensitivity of cellular targets to metal ions. A single metal or a group of metals with compounds related to them may be subject to one or more detoxification pathways. However, the type of microbes will have a significant impact on the mechanisms for detoxification (Shoeb et al., 2012). Ojuederie and Babalola (2017) also discuss the ability of microorganism enzymes to more quickly and effectively digest harmful metals, highlighting new developments in phytoremediation and microorganism bioremediation for the removal of harmful metals from the environment. To ensure the protection of the environment, biotechnological initiatives should, however, strictly comply to safety regulations.

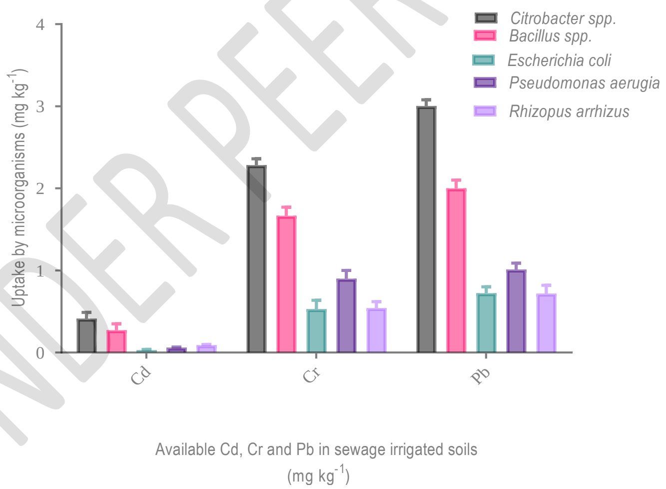

Fig. 3: Bioaccumulation of Cd, Cr and Pb by microorganisms in sewage irrigated soils (dry weight soil)

Bioaccumulation of heavy metals by microorganisms: The available heavy metals in the sewage-irrigated soils were Cd (3.15±0.03 mg kg^{-1}), Cr (6.35±0.07 mg kg^{-1}) and Pb (7.15±0.09 mg kg^{-1}) (Table 1).The microorganisms grown in metal-enriched sewage irrigated soil took up metal ions in varying degrees. The biological detoxification system was largely influenced by the availability of metals in terms of metal uptake. Through *Citrobacter spp*., the uptake of Cd was 13.2%, Cr 36% and Pb 42% which resulted in the maximum amelioration of the sewage irrigated soils. (Gomaa and El-Meihy, 2019) reported that *Citrobacter freundii* MG812314.1 isolated from Al-Rahawy drain was found to be the foremost potent biosurfactant producer. The made biosurfactant exhibited a good capability to detoxification of the heavy metals reckoning on the factors like time and concentration of inoculums used. Through *Bacillus spp*., the uptake of Cd was 8.6%, Cr 26% and Pb 28% which resulted in the second highest amelioration of the heavy metals contaminated sewage-irrigated soils. However, the uptake heavy metals by Escherichia coli and *Pseudomonas aeruginasa* were observed marginal in the sewage-irrigated soils. The uptake by *Rhizopus arrhizus* of cadmium 2.98%, chromium 8.5% and 10.2% decreases in sewage irrigated soils. Which grown in all microorganisms have applied in *Citrobacter spp.* and *Escherichia coli* highest and lowest accumulated of Cd, Cr and Pb in the sewage-irrigated soils. The order of accumulation of the heavy metals was Pb > Cr > Cd in the sewage-irrigated soils (Fig. 3). Biosorption, bioaccumulation, biotransformation, and biomineralisation are the techniques utilized by microorganisms for his or her continued existence within the metal-polluted surroundings. Significant metal removal is dole out by living organisms or dead biological materials. (Roell et al., 2019) Optimising strain inoculations, organic process divergence and crossing feeding, the development of mutualistic growth, cell immobilisation, and biosensors may all be used to regulate cell populations in order to create stable consortia. Large-scale practicality applications or biosorption procedures have demonstrated that dead biomass is more useful than the bioaccumulation strategy, which makes use of living organisms and so necessitates nutrient supply and sophisticated bioreactor systems. The inability to maintain a healthy microbe population will also be impacted by the toxicity of contaminants, in addition to other unfavourable environmental factors. A genuine attempt is made to present various aspects of metal pollution in soils in order to understand the significance and risk associated with important metals. Additionally, the beneficial qualities of important metals and their toxicity to plants and microbes are discussed. Biological systems utilise a variety of strategies to detoxify important metals, and these strategies are significantly underlined in the last sentence (Oves et al., 2016). Many of the distinctive qualities of living microbes haven't, however, been used in large-scale applications. Because it comes into contact with large metal waste material to achieve the purpose of rectification, the selection organism should develop resistance to metal ions. The organisms of selection are isolated from different environments and dumped at the contaminated location, or they are endemic to the contaminated environment.

Comments on detoxification strategy: Numerous microorganisms have already developed defences against environmental stress, and these wonderful creatures might be used effectively to clean up seriously metal-contaminated soils. The fundamental benefit of microbic rectification is that it has a significant economic advantage over alternative methods. Currently, techniques for engineering microorganisms for efficient serious metal rectification are being gradually transferred from the fundamental knowledge of organic chemistry and metabolic pathways involved in serious metal resistance. (Frederick et al., 2013) Engineered microorganisms to supply trehalose and establish that it reduces one millimeter metallic element (VI) to metallic element (III). Built *Chlamydomonasreinhardtii* generated a major increase in tolerance to Cd toxicity and its accumulation (Ibuot et al., 2017). Genetically built microbes for serious metal rectification involve the employment of *Escherichia coli* (*E. coli* ArsR (ELP153AR)) to focus on As(III) (Kostal et al., 2004) and yeast (CP_2 HP_3) to focus on Cd^{+2} and Zn^{+2} (Vinopal et al., 2007). Chemical interactions mediated by microorganisms, such as reduction reactions, may result in less mobile heavy metal and mixed solid phases capable of sorbing serious metal, improving the processes of immobilisation. Although the mechanism of immobilisation by action is reversible, the remobilization of ice may happen if the biogeochemical circumstances of the area change over time. Utilising living or dead biomass and its components, microbic biosorption aims to remove and recover metals and metalloids from solutions. highlights the teams of microorganisms with biosorbent capability for serious metal removal and discusses the sources of poisonous significant metals (Ayangbenro and Babalola, 2017).

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Microorganisms have developed resistance systems to almost all harmful metals as a result of selective pressure from an environment rich in metals. The biosorption capability relates to the removal of heavy metals via bacteria, fungi, biofilm, algae, genetically modified microbes, and immobilised microbial cells. As a sustainable environmental technology in the near future, the application of biofilm has demonstrated synergistic effects with a multifold increase in the removal of heavy metals (Ramrez Calderón et al., 2020). A cell may create metal defence mechanisms in an effort to safeguard delicate cellular components. Cellular components can be made less sensitive to metals by modifying them or restricting their availability to metals. The mechanisms involved in producing their toxicity in order to highlight the need for the creation of methods to reduce exposure to these metals and to identify compounds that significantly help to counteract their harmful effects within the bodies of living organisms (Jan et al., 2015). The kind and number of metal absorption mechanisms, the function of each metal in regular metabolism, and the existence of genes that regulate metal resistance on plasmids, chromosomes, or transposons are all variables that affect how much resistance an organism has to metals. Because this green remedy method is low-cost and takes advantage of plant and rhizosphere microorganisms, microbially enhanced phytoextraction has greater potential for cleaning up heavy metal-contaminated soil.

Conclusion

Independent of the soil type, the distribution pattern of the heavy metals in the soil profile is often uniform. Lead, cadmium, and chromium content, however, is higher near the surface horizons and dramatically declines with depth. Because of the quick weathering of carbonate minerals, there is a substantial relative enrichment of heavy metals in these soils. Most likely, the profile of Jhunsi, which is moderately contaminated on both profiles, is defined by the sedimentation of eroded topsoil in the depression. The increased concentration of air pollutants was the source of the higher lead buildup. Pb levels may be rising as a result of automobile emissions. Due to the release of industrial waste from the dye industry, tanneries, and batteries, the Cr and Cd concentration increased. Due to the greater levels of metal adsorbent phases, the Naini profile, which absorbed somewhat more sewage-sludge than the other soil types in the research area, is distinguished by having higher heavy metal contents. The amount of soil organic carbon rose and soil fertility increased when sewage water was used for irrigation over an extended period of time. However, periodic monitoring of metal accumulations in soil must be conducted strictly. In soil, heavy metals precipitate as hydroxide, carbonates, sulphides, phosphates, and silicates after reacting with organic matter, clay exchange sites, carbonate, and oxide surfaces. It is also thought that a common mechanism for reducing the risks associated with contaminated soils is metal immobilisation by precipitation and adsorption.

The heavy metal pollution can be dealt with by the activities of microorganisms through heavy metal detoxification. Among the tested microorganisms, the highest accumulation of heavy metals in the sewage irrigated soils was observed in *Citrobacter spp.* to the extent of Cd 13.2%, Cr 36%, and Pb 42%. However, the lowest accumulation was observed in *Escherichia coli* to the extent of Cd 1.2%, Cr 8.4%, and Pb 10.2%. Among the tested heavy metals, the order of accumulation was Pb > Cr > Cd. The present study indicates a significant reduction of heavy metals from the contaminated soils through the bioremediation process. The future answer for the detoxification of heavy metals in soils may depend on interactions between the detoxifying microorganisms and the extent of phytoremediation helped by microbes. In-depth research and long-term, ongoing monitoring are required to make sure that the biological conditions in the soil are ideal for the proper operation of the microbes to reduce the toxicity of heavy metals. To increase cleanup rates and efficiency, engineering strategies utilising environmentally friendly goods may be taken into account.

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