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Biosurfactants : A Novel Approach to Manage Heavy Metals Contamination in Soil - A Review

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ABSTRACT

Heavy metal contamination in soil is a critical environmental issue that poses significant risks to human health, agricultural productivity and ecosystem stability. Traditional remediation techniques often fall short due to their high cost, low efficiency and potential secondary pollution. Biosurfactants, microbial-derived surface-active compounds, offer a novel and sustainable approach to address this problem. These compounds increase the bioavailability and enhance the mobility of heavy metals, facilitating their removal or stabilization in contaminated soils. Biosurfactants offer several advantages over conventional methods, including biodegradability, low toxicity and effectiveness at low concentrations. Their production from renewable resources and potential for *in situ* application further underscores their environmental and economic benefits. This review explores the properties of biosurfactants, mechanisms by which biosurfactants interact with heavy metals, different biosurfactants employed in soil remediation, their effects on soil properties, effects on plant growth, its limitations, comparison with synthetic surfactants and available commercial products. By integrating biosurfactants into heavy metal remediation strategies, it is possible to achieve more sustainable and efficient management of contaminated sites.

Keywords : Heavy metals, Soil contamination, Biosurfactants, Surface tension, Soil washing, Remediation

ACCORDING to the Central Pollution Control Board (CPCB), a site is considered contaminated when it contains hazardous pollutants at levels that threaten human health and the environment. Of 240 sites in 21 Indian states, 127 are contaminated and 113 are probable contaminated sites (CPCB, 2022). Heavy metal contamination, involving elements like lead (Pb), cadmium (Cd), mercury (Hg) and arsenic (As), is a significant environmental issue. These metals, which persist in the environment, can accumulate in plants and animals, leading to various levels of toxicity depending on exposure and dose (Mansourri *et al*., 2016). Sources of contamination include industrial processes, mining and improper waste disposal. Studies have also revealed that increased use of fertilizers and plant protection chemicals in crop cultivation has resulted in increased concentrations of chemical residues and heavy metals in the soil causing pollution (Uday *et al.,* 2022). These heavy metals impact plant health very negatively (Hamsa and Prakash, 2018).

Soil remediation may start by looking up biosurfactants produced by microorganisms such as bacteria and fungi as a potent solution. These agents are biodegradable and eco-friendly, enhancing the solubility and mobility of heavy metals thus facilitating the desorption of these elements from soil particles to make extraction easier or even stabilization (Banat *et al*., 2010). Unlike synthetic surfactants, biosurfactants pose minimal risk of secondary pollution and can work under different environmental conditions including variations in pH value, temperature and salt concentration. A great deal of

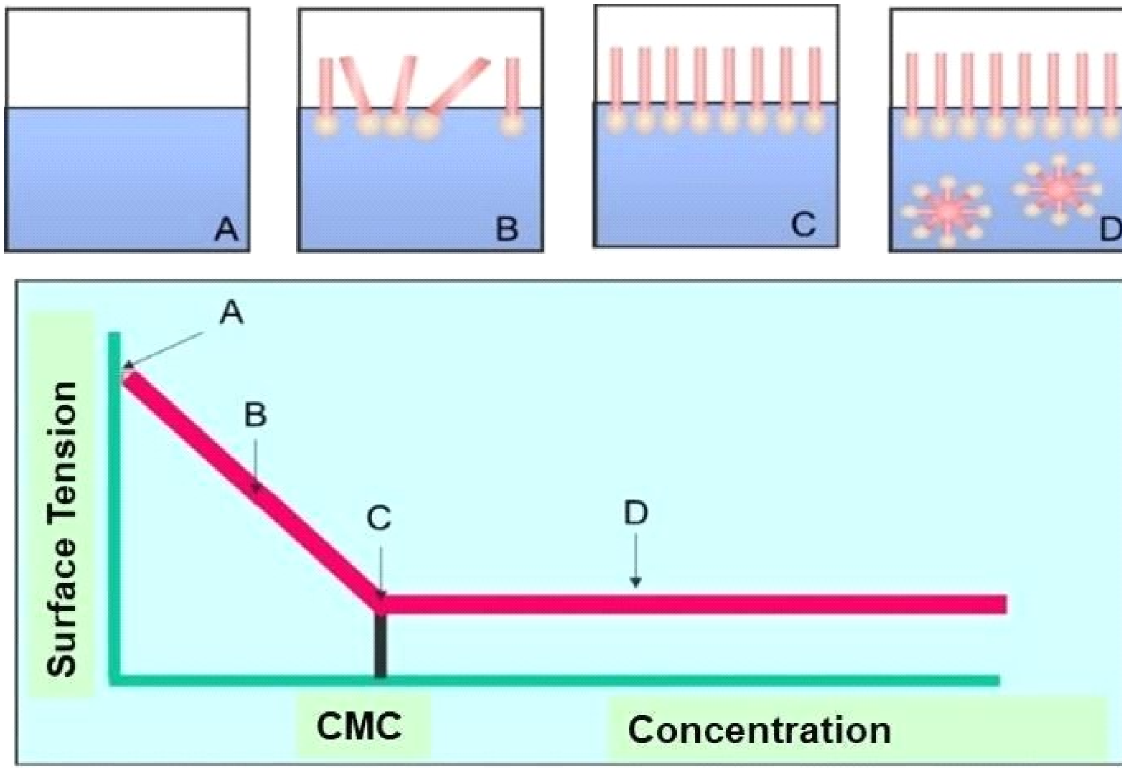

Fig. 1 : Micelle formation at Critical micelle concentration (Santos *et al.,* 2016)

research has been done on biosurfactants because they may be good candidates for effective and environment-friendly techniques for rehabilitating polluted lands (Rosa *et al*., 2015; Pacwa-Plociniczak *et al*., 2011; Makkar *et al*., 2011 and Vijayakumar *et al*., 2015).

Properties of Biosurfactants

Biosurfactants tend to assemble due to the presence of Van der Waal forces and Hydrogen bonding to form micelles at their critical micelle concentration. This micelle formation property of biosurfactants can be affected by various external factors. Jahan *et al*. (2020) suggested that changes in biosurfactant concentration, solution salt content, temperature, pH and pressure, etc. can modulate the micelles' size and shape.

They significantly lower the surface tension of water and the interfacial tension between immiscible fluids like oil and water. When the rhamnolipid concentration was increased from zero to 40 mg L^{-1}, the surface tension of water was decreased from 72 to 35 mN/m. With a further increase in the rhamnolipid concentration to 200 mg L^{-1}, the surface tension was reduced to 28 mN/m, as reported by Haryanto and Chang (2015). Also, they are naturally occurring compounds and so, more biodegradable than synthetic surfactants, hence less toxic which reduces adverse environmental impacts.

Many biosurfactants are stable across a wide range of temperatures and pH levels, which is advantageous for various industrial processes. Rocha *et al*., 2018 studied the effect of pH and temperature on surface tension reduction capacity. They found that there are no significant changes over a wide range of pH and temperature. Similar results were found by Kim *et al*., 2000 and Franca *et al*., 2015. In contrast, Saponin exhibits stability only under alkaline conditions (Liu *et al*., 2015). This suggests that the suitability of the biosurfactants can be decided based on the prevailing conditions.

One more important property of biosurfactants is their HLB number. HLB stands for hydrophilic-lipophilic balance. This number describes the relation between

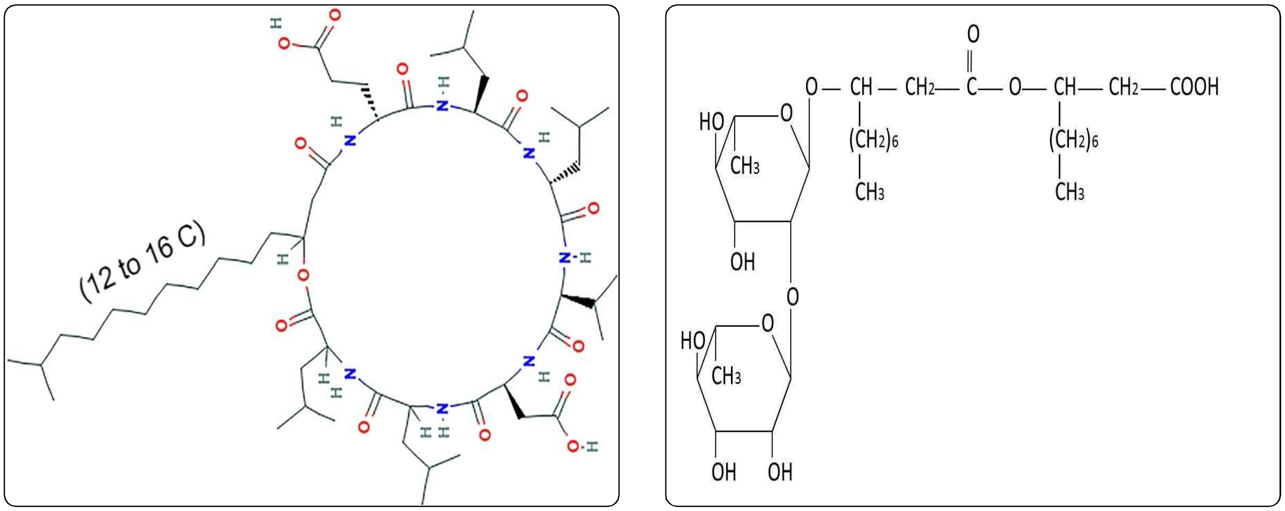

Fig. 2 : Chemical structure of a) surfactin (Zanotto *et al.,* 2019) and b) Rhamnolipid (Kapadia *et al.,* 2013)

the hydrophilic and hydrophobic parts of the molecule. It is used to decide which biosurfactant will be suitable for use. A study by Schmidts *et al.* (2010) suggests that a biosurfactant with a HLB value between 3 to 8 forms water in oil emulsion (w/o systems) and that between 9 to 12 form oil in water emulsion (o/w systems). A higher HLB value will suggest higher water solubility.

Production of Biosurfactants

Biosurfactants production involves the use of plant as well as microbial sources. It is usually produced extracellularly or as part of the cell membrane by yeast, bacteria or filamentous fungi. *Bacillus subtilis* MTCC 2423 strain grown on nutrient agar media was used to prepare biosurfactant by Jayalatha *et al*. (2024). *Lactobacilli* strains referred to as Generally Recognised as Safe (GRAS) strains, in contrast to the biosurfactants produced by pathogenic strains, e.g.

TABLE 1

Microbial origin and HLB number of different biosurfactants (Sharma *et al.,* 2023)

Biosurfactant	Microbial origin	HLB number
Rhamnolipids	*Pseudomonas aeruginosa*	10.17
Lipopeptides	*Bacillus cereus*	10-11.1
Sophorolipids	*Candida bombicola*	10-13
Surfactin	*Bacillus subtilis*	10-12
Glycolipids	*Candida apicola*	10-15

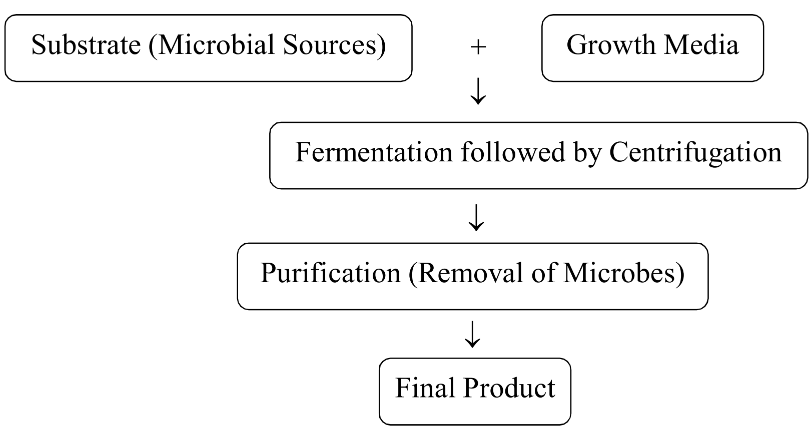

Fig. 3 : General procedure of biosurfactant production

Pseudomonas sp. have also been used for production purposes (Patowary *et al.,* 2016; Samykannu and Achary 2017 and Jimoh & Lin, 2020). It signifies that various strains can be assessed for their potential to be used for the production of biosurfactants. However, at the same time, there are no standardized procedures for the production of commercial products of biosurfactants.

Verma *et al.,* 2022 have attempted biosurfactant production in bacteria isolated from Oil and Pesticide-contaminated soil of Ranchi district, India. Thereby, suggesting that their production can also be done by employing contaminated sites.

At the same time, they can be obtained from plant sources. Some of the biosurfactants like saponins, phospholipids or lecithin are released by plants. They are widely distributed in nature and out of them saponin is the most dominant. Steroidal saponins are extracted from plants belonging to monocot families like *Agavaceae, Asteraceae, Balanitaceae, Costaceae, Dioscoreaceae, Leguminosae, Liliaceae, Ruscaceae, Solanaceae*, etc., while triterpene saponins originate from mostly dicotyledonous families like *Leguminosae, Araliaceae* and *Caryophyllaceae* (Sparg *et al.,* 2004, Zhou *et al.,* 2013). The plant-derived surfactants are being paid more attention due to the possibility of more yield than the microbial surfactants (Du *et al.,* 2020).

TABLE 2

Plant species that are used as source of biosurfactants

Plant species	References
Chenopodium quinoa, Glycine max, Malpighia emarginata	Bezerra *et al.,* 2021
Acacia concinna, Citrus aurantifolia, Zizypus spina-christi, Phyllanthus emblica	Badi and Khan, 2014
Medicago sativa, Saponaria officinalis, Smilax regelli	Liu *et al.,* 2017

Removal of Heavy Metals Using Biosurfactants

Methods employed for removal: The action of biosurfactants to remove heavy metals or any other pollutants is carried out by two types of operations: soil washing and soil flushing. Soil washing is a remediation process used to treat contaminated soil by physically separating contaminants from soil particles. It involves using water, sometimes with added chemicals, to 'wash' the soil and remove hazardous substances such as heavy metals, hydrocarbons and other pollutants. The process works by exploiting differences in the physical or chemical properties of contaminants and soil particles, such as solubility or particle size. Soil washing is a technique that can permanently remove heavy metals from the soil, despite concerns about nutrient loss and secondary pollution (Wei *et al*., 2016 and Feng *et al*., 2020). Soil flushing is an *in-situ* technique involving the injection of biosurfactants into the contaminated soil for treatment and making use of drainage pipes. However, soil flushing is not very feasible for small farmers. They require skills as well as infrastructure for its employment. These techniques are popular in countries like the Netherlands, the US and Europe. But the cost factor sometimes becomes the cause of its less use.

Removal mechanism : The main thing to understand here is how biosurfactants are initiating their remediation action. Compared to synthetic surfactants, biosurfactants often have more functional groups and larger molecular structures, which provide biosurfactants with extraordinary surface activity for the extraction of hydrophobic organics and heavy metals (Chen *et al.,* 2015). The processes of ion exchange, precipitation-dissolution and counter-ion complexation are potential methods by which biosurfactants can extract heavy metals. Biosurfactants can lessen interfacial tension and improve the solubility and bioavailability of hydrophobic organic substances through the creation of micelles (Santos *et al.,* 2016 and Amani, 2018). They are known to cause emulsification (formation of micelles) which increases the contact surface area of the pollutants which leads to easier transport of them towards the aqueous phase.

How much concentration and which biosurfactant can be used : The specificity of the heavy metal or the biosurfactant can be based on the removal efficiencies of the individual biosurfactants as mentioned in Table 3. The effective concentration of the biosurfactants ranges from 0.5 to 5 per cent, which varies with soil type, organic matter content, etc. The highest removal of heavy metals has been reported at the critical micelle concentration (CMC) of the biosurfactant (Rocha *et al.,* 2018).

Removal per cent of different biosurfactants : As stated by Sarubbo *et al.* (2018); heavy metal removal is reported to be greatly aided by biosurfactants. Using 0.8 per cent surfactant solution resulted in the removal of 99.9, 96.8 and 93.5 per cent of Fe, Pb and Zn, respectively. From the polluted soil 33.23 per cent, 27.46 per cent and 45.85 per cent of Ni, Cr and Cd were removed by washing (Kholghi *et al.,* 2020). Similar results were reported by Haryanto and Chang (2015), who discovered that batch washing, flushing, and foam-enhanced removal eliminated around 32, 12, and 25 per cent of inner interaction type and 45, 23 and 49 per cent of outer sphere/remaining type Cu ions, respectively. The greater interaction of the inner sphere type Cu ions was thought to be the cause of the discrepancy between the elimination of inner interaction and residual type Cu ions. These findings certainly suggest the efficacy of biosurfactants in heavy metal removal action. However, they do not suggest the suitability of the types of biosurfactants as well as the efficacy of individual biosurfactants as we can observe that there is variation in the removal per cent of the heavy metals.

Number of Washings Required for Efficient Heavy Metal Removal

It might be possible that all the heavy metals are not removed at once *i.e.*, a single washing might not provide satisfactory results. According to Sarubbo *et al.* (2015), we need to perform multiple washings to efficiently remove heavy metals as there is a strong interaction between soil and metals. After five washings, the removal rates increased to 100 and 50 per cent for Zn and Cu, respectively from initial values of 16 and 37 per cent for Zn and Cu, respectively, obtained by Mulligan *et al.* (2001). In the same instance, Rocha *et al.,* 2018 found that a single washing was sufficient to remove almost all the Cu and Pb ions and the second washing increased the removal by 9 per cent. So, we can assume that multiple washing is better for effective and coherent removal. However, Gusiatin *et al.* (2019) add that sequential soil washing is more efficient with the combination of different biosurfactants (SAP-RAM, TAN-RAM).

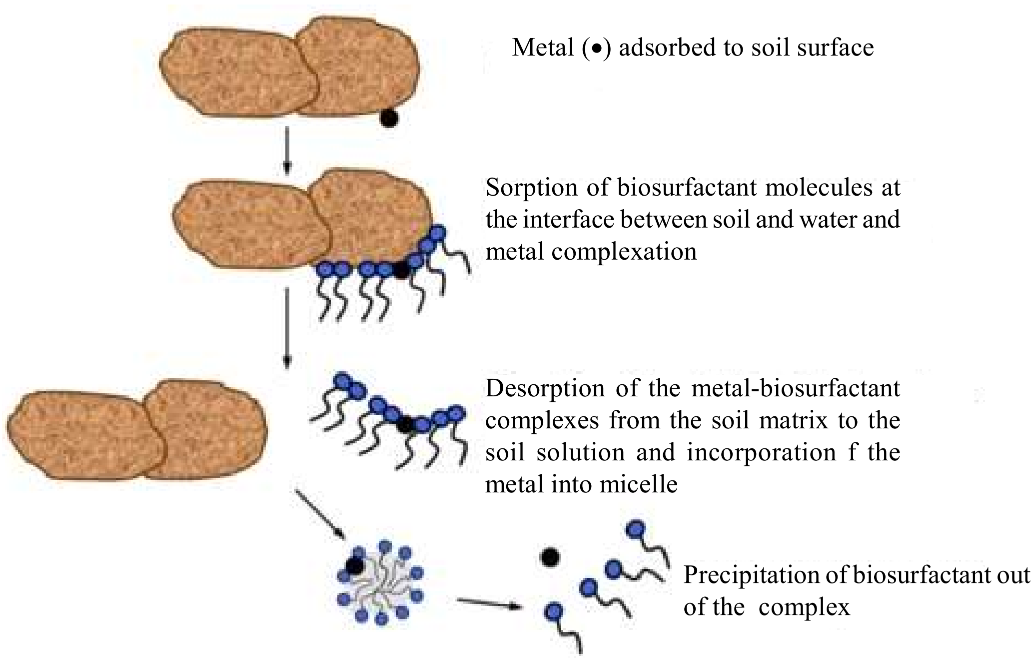

Fig. 4 : Mechanism of Biosurfactant activity in contaminated soil (Pacwa-Plociniczak *et al.,* 2011)

TABLE 3

Different biosurfactants used in heavy metal removal in soil

Biosurfactant	Process	Heavy metal removed	References
Rhamnolipid	Soil Washing	Cu (65%) Zn (18%)	Mulligan *et al.* (2001)
Rhamnolipid	Soil Flushing	As, Cu, Zn, and Pb	Wang and Mulligan (2009)
Lipopeptide	Soil Washing	Pb (69%) Cd (54%) Cr (43%)	Ayangbenro and Babalola (2020)
Surfactin	Soil Washing	Cu (15%) Zn (6%)	Mulligan *etal.* (2001)
Sophorolipid	Soil Washing	As (91%)	Arab and Mulligan (2018)

This shows that multiple washing along with the combination of biosurfactants offer greater removal. Some researchers suggest multiple washings and at the same time, some have obtained efficient removal in a single washing. It means that there is a need for more study this aspect will affect the economic feasibility of the products.

The contaminants are removed along with the washing solution. As biosurfactants are reusable, they can be recovered for further use. This suggests that it can also be a cost-effective option. Rocha *et al.* (2018) have mentioned the cost-efficient use of biosurfactants where biosurfactant was itself used as the washing solution. However, some researchers report that due to the labile nature of biosurfactants, they cannot be reused (Christofi and Ivshina, 2002). This contrast gives rise to the need for more work on stabilized formulations of biosurfactants.

TABLE 4

Heavy metal removal efficiency from sand based on the number of washings with solutions of crude biosurfactant from *C. tropicalis* (Rocha *et al.*, 2018)

Washing with Solutions of crude biosurfactant	Removal (%)		
	Zn	Cu	Pb
1st Washing	51 ± 1.1	56 ± 1.2	10 ± 1.5
2nd Washing	9.4 ± 0.5	3.9 ± 0.7	0.99 ± 1.0
3rd Washing	1.2 ± 0.3	0.6 ± 0.2	0.46 ± 0.3
4th Washing	0.27 ± 0.1	0.2 ± 0.1	0.36 ± 0.1
5th Washing	0.17 ± 0.1	0.2 ± 0.2	0.25 ± 0.3
Total	62.04	59.9	12.06

Effect of Biosurfactants on Soil Properties

To consider any management strategy for heavy metals polluted soil, we must consider its various after-effects on the soil properties. Hence, we will be delving into the different effects of biosurfactants on soil properties. Biosurfactants are found to affect soil's physical, chemical as well as biological properties. Regarding the physical properties, biosurfactants decrease soil permeability which may occur due to the dispersion of colloids and dissolution of carbonates and oxides. As reported in some studies, they lead to a slight decrease in hydraulic conductivity which may be due to the clogging of pores and increase in viscosity of the medium. Also, as per the sorption coefficients of kaolinite clay: biosurfactant > SDS > naphthalene > Triton X-100, biosurfactants have the highest power of lubricating the soil particles. This gives soil the densest configuration (Park *et al.,* 2006). Water holding capacity also increases in biosurfactant-remediated soil (Guo and Wen, 2021). This means that overall, it improves soil physical properties.

Biosurfactants impact the chemical properties of soil. They cause a reduction in soil pH. Microbial degradation as enhanced by biosurfactants leads to a decrease in the pH of soil. The organic matter content of soil decreases in the presence of biosurfactants that can be attributed to the enhancement of bioavailable nutrients for cultivated plants through the mineralization of organic matter. Biosurfactants on decreasing the surface tension of compounds, increase the mineralization of soil organic matter, according to Singh *et al.,* 2020. The total organic carbon (TOC) also decreases at the end of the bioremediation process which might be due to microbial degradation. The total organic carbon (TOC) of the soil showed a decrease from their initial values when different concentrations of biosurfactant were applied in the bioremediation (Guo and Wen, 2021).

As they increase the bioavailability of many organic compounds or contaminants present in soil, they ease the microbial degradation activity. Also, biosurfactants itself act as carbon source for microbes which might be the reason of increase in the microbial activity. Guo andWen (2021) reported that microbial count expressed in colony-forming units (CFU) per gram of soil increased in biosurfactant-treated soil. The microbial quantity in the biosurfactant-treated sample reached 7.2×10^8 and 6.5×10^9 CFU/g-soil at biosurfactant concentrations of 0.3 % and 0.5 % (w/w), respectively from 4.5×10^6 CFU/g-soil, at the remediation time point of day 60. After that, there was a decrease in the microbial count which may be attributed to the fact that the hydrocarbons solubilized by the biosurfactants got depleted in soil. They can also promote the earthworm population (Shi *et al.* 2020).

The dehydrogenase activity of soil also increases by the application of biosurfactants. In the absence of biosurfactants, there has been an increase in dehydrogenase activity by 10.9 per cent while in their presence it was 28.7-81.2 per cent (Guo and Wen 2021). Also, measurements of dehydrogenase activity on the application of biosurfactants have suggested that soil microcosms can tolerate biosurfactants up to a limit without any adverse effects *i.e.,* 4 g L^{-1} rhamnolipids according to Millioli *et al.* (2009).

The findings have focused mostly on the positive effects of the application of biosurfactants on soil properties. However, as the procedure of washing might lead to nutrient losses (Wei *et al.,* 2016), more studies need to be done on the effects on the nutrient (N, P_2O_5, K_2O, etc.) contents of soil as it will be a key factor in describing its feasibility for the management of soil pollution.

Biosurfactants and Plant Growth

Many researchers have carried out their study on how the application of biosurfactants affects plants. They have reported enhanced germination (Singh and Rathore, 2019), decreased nutrient stress (Nguyen and Marschner, 2017) and decreased toxicity. Biosurfactants increase microbial activity which in turn leads to an increase in soil respiration. This increased soil respiration can be considered a cause of many favorable things for plants like the removal of pollutants and increased nutrient availability.

The favorable effects of biosurfactants are observed in the germination phase or cell division phase (Singh and Rathore, 2019). They help to enhance plant growth in this manner. Considering these positive effects, it can be said that biosurfactants can be employed in phytoremediation also where it can be used to enhance plant growth.

Limitations in the Use of Biosurfactants

Some of the countries have employed the soil-washing procedure. The commercial use of biosurfactants is however quite limited due to the need for large infrastructure and trained personnel to carry out the washing or flushing procedure. Also, many projects were discontinued due to the cost involved and ineffectiveness at varying soil conditions (Dermont *et al.,* 2008). The production process of biosurfactants has also been a constraint in making use of biosurfactants. Many scientists have attempted to minimize the cost factor. For example, Rocha *et al.,* 2018 made use of crude biosurfactant as substrate for the production of biosurfactants that reduced 20-30 per cent of the production costs. So, there are many ways to work out these limitations. We can eliminate the purification step, use raw materials efficiently and utilize biosurfactant solution itself as the washing solution to reduce the cost.

Biosurfactants v/s Synthetic Surfactants

The biosurfactants have shown more advantages as compared to synthetic surfactants like sodium lauryl sulphate, sodium stearate, etc. Properties like biodegradability, micelle formation, stability under different conditions of pH, temperature and salinity and the ability to reduce surface tension make them more feasible for use in various fields. Many works have been carried out to compare both of them.

The critical micelle concentration (CMC value) of a biosurfactant ranges between 1-200 mg L^{-1} which is on par with the synthetic ones (Nitschke *et al.,* 2011). Biosurfactants are found to be more effective in reducing surface tension than synthetic surfactants (Vaz *et al.*, 2012).

The toxicity of biosurfactants have also been assessed and it has been found that they are less toxic than the synthetic ones. The impact on seed germination was tested by Santos *et al.,* 2017 and they observed that biosurfactants derived from *Candida lipolytica* had no impact on seed germination.

Comparison of their ability to carry out soil washing for the removal of pollutants has shown that biosurfactants are more efficient than the synthetic surfactants which might be due to them being more effective in reducing interfacial tension. They can also be preferred more due to their biodegradable nature. Amani *et al.,* 2015 demonstrated that the biosurfactants and surfactants are useful for the sand washing with removal percentages of 80, 77, 65 and 61 per cent at the room temperature for rhamnolipid, surfactin, Triton X-100, and SDS, respectively.

So, we can assume that biosurfactants have more to offer than chemically synthesized surfactants.

Market Availability of Commercial Products

The market for biosurfactants has been steadily growing, driven by increasing consumer demand for sustainable and environmentally friendly products across various industries. The cost of biosurfactants ranges from 1000-2000 rupees per 500ml bottle. India is expected to have a rise in the market of biosurfactants, as government policies are also focusing on environment-friendly products. Market sources assume that biosurfactants will reach a compound annual growth rate of 5-6 per cent by the year 2029. Some commercially available products are-

- Rhamnolipids are currently available from Jeneil Biosurfactant Inc. (USA), Ecover (France) and Rhamnolipid Participations Inc. (USA), where as sophorolipids (lipid portion linked to sophorose-reducing sugar) are offered as Sophoron TM by Saraya (Japan) and Soliance (France) (Rocha *et al.*, 2018).
- SAP (product no. 84510) is a non-ionic biosurfactant in the form of a powder, with a sapogenin content of 8–25 per cent (Sigma-Aldrich). TAN (product no. 16201) is a natural polyphenolic compound. (Gusiatin *et al.,* 2019). MSI 54 is a lipopeptide biosurfactant.
- Other products are Tween 80, Tergitol 15S9 and Triton-X-100. These are non-ionic surfactants (Rocha e Silva *et al.,* 2018).

Biosurfactants seem to be an upcoming promising agent for remediating polluted soil. They increase the bioavailability of pollutants or hydrocarbons in soil and enhance their removal.They have also been recognized for improving certain soil properties. They increase nutrient availability and increase microbial population. Also, they are biodegradable which increases its acceptance as a washing agent. Soil washing has come up as a very good substitute for landfilling or stabilization processes for heavy metals management (Dermont *et al.,* 2008). Moreover, there have been many works regarding the different methods of application *i.e.*, single washing and sequential washing. The treatment of multi-HM-contaminated soils, sequential soil washing with plant biosurfactants and then with microbial rhamnolipids could remove metals more efficiently than a single washing or sequential washing employing the same class of biosurfactant in each step (Gusiatin *et al.,* 2019). This means that the efficiency can vary with the method employed for remediation. One type of biosurfactant may not be suitable for use for all heavy metals.

Despite the environmentally friendly characteristics and biodegradability of these compounds, the production process remains a constraint to the commercialization of biosurfactants. There is not yet a sufficient economic technology for the recovery and purification of biosurfactants on a large scale (Bezerra *et al.,* 2018).

Future Line of Work

- To find potential cost-effective sources of biosurfactants.
- To optimize the process of use of biosurfactants for the management of heavy metals in soil.
- To improve the method of soil washing to reduce cost.

- Studies on field applicability.
- To study the chemistry related to the structures of biosurfactants and their interaction with soil.

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