Chapter I:

INTRODUCTION

1.1 General

Water is one of the most essential resources needed for the survival of life and development (Noor et al., 2023). Approximately 71% of the earth's surface is covered with water, of which 96.5% is in oceans. Of the total water, only 2.5% is freshwater, with 1.2% being surface water and around 0.8% groundwater that serves all life forms (WSS, 2019). However, population growth, climate change and industrialization have worsened the water quality owing to the production of various pollutants (Elbeltagi et al., 2022; Zamora-Ledezma et al., 2021). Some of the major contaminants of concern include pharmaceutical products, biocides, plastics, nanomaterials, heavy metal(loid)s and organic matter (Villarín and Merel, 2020), with heavy metal(loid)s being among the most released pollutants (Azimi et al., 2017). Elements with relatively high density (at least five times greater than that of water) and toxic even in trace amounts are termed as heavy metal(loid)s (Gaur et al., 2021). Such elements include arsenic, cadmium, chromium, copper, lead, mercury, nickel, etc. (Gautam et al., 2014). Presence of heavy metal(loid)s in water is a cause of global concern (Anjana et al., 2007).

Pollution of heavy metal(loid)s in water can be caused by either natural or anthropogenic sources (Hansda et al., 2016; Sekomo et al., 2012; Sharma et al., 2021). Natural sources include rocks containing mineral ores, volcanic eruptions, wind-blown dust particles and forest fires. Anthropogenic sources include industrial activities (such as burning fossil fuels), domestic and agricultural activities, mining-related activities. Other miscellaneous sources may consist of incineration of landfills and open dumps, traffic emissions, e-wastes and biomedical wastes (Gautam et al., 2014; Hait and Tare, 2012; Priya and Hait, 2018; Srivastava et al., 2017; Zamora-Ledezma et al., 2021). In some regions, up to 60% of groundwater is contaminated with geogenic groundwater contaminants including arsenic, fluoride, selenium and uranium with their concentrations being above the permissible drinking water levels (Mukherjee et al., 2024). Heavy metal(loid)s are non-biodegradable and have bioaccumulating properties, which makes them potentially hazardous (Banerjee et al., 2015). They also have high toxicity and lead to adverse effects on human and environmental health (Hlihor et al., 2014; Muthu et al., 2019; Sevak and Pushkar, 2024). Arsenic is one such heavy metalloid that is ranked number one on the Agency for Toxic Substances and Disease Registry's (ATSDR) substance priority list (ATSDR, 2023).

1.2 Arsenic

Along with nitrogen, phosphorous, bismuth and antimony, arsenic belongs to Group 15 of the periodic table and is a metalloid. Arsenic ranks as the 20th most abundant element in the

Earth's crust and the 12th most abundant in the human body (Askenaizer, 2003; Raj and Maiti, 2020; Reis and Duarte, 2019). Since its outermost electrons are fixed by covalent bonds, arsenic rarely occurs in a free state. General properties of arsenic are listed in Table 1.1 (Rahman et al., 2024). It occurs in various oxidation states, from +5 to −3, with arsenate [As(V)] and arsenite [As(III)] being most prevalent. Oxidation states of arsenic is greatly affected by pH and redox conditions (Liu et al., 2020). Owing to its higher solubility, As(III) is more toxic (~60 times) as compared As(V) (Sattar et al., 2019; Verma and Sinha, 2023). In aqueous environments, pH plays an important part in the speciation of arsenic. As(III) exists in the forms of $As(OH)_3$, $As(OH)_4^-$, AsO_2OH^{2-} and AsO^{3-} whereas As(V) in the forms of AsO_4^{3-}, $HAsO_4^{2-}$ and $H_2AsO_4^-$ in the pH range 3 to 11 (Arabi et al., 2021; Rahman et al., 2024). Methylated forms of arsenic may result due to microbial actions (Jia et al., 2013).

Organic species include arsenobetaine, arsenocholine, dimethyl arsinate [DMA(V)], dimethyl arsenite [DMA(III)], monomethyl arsenate [MMA(V)], monomethyl arsenite [MMA(III)], trimethyl arsine oxide (TMAO) and arsenosugars (García-Vargas and Cebrián, 2023). There are more than 300 different species of arsenic (Zhang et al., 2022). Organic arsenic (*o*As) has a framework of carbon atoms, whereas inorganic arsenic (*i*As) compounds do not. *o*As compounds have complex structures but are less toxic than *i*As compounds. *i*As can be up to ten times more toxic than *o*As (Garland, 2007).

1.3 Sources and distribution

Arsenic in the environment can be a result of both natural as well as anthropogenic sources (Aryan et al., 2024). Natural sources include rock and soil erosion, volcanic eruptions and weathering (Bundschuh et al., 2021), whereas common anthropogenic sources are burning of fossil fuels, mining, wood preservatives, arsenical herbicides, fungicides and insecticides (Nriagu et al., 2007). Arsenic is distributed in the earth's crust, sediments, soil, air, water and biological life forms. The distribution of arsenic in the atmosphere is uneven in the northern and southern hemispheres. Of the total arsenic, 85% exists in the northern hemisphere, whereas only 15% is in the southern hemisphere (Masuda, 2018).

Particulate matters in the air have high concentrations of arsenic, with a maximum reported concentration of 1660 ng/m^3, industrial effluents, pesticides, etc., are sources of arsenic in soil with a maximum concentration of 4600 mg/kg, whereas arsenic in sediments can be a result of their reservoirs with maximum concentration reaching up to 4500 mg/kg (Patel et al., 2023). Arsenic concentrations of 100 to 5000 µg/L in ground and surface water have been reported near sulfide mineralization areas and smelting sites, whereas in hydrothermal systems, the range is between 1386 to 5850 µg/L (Zhang et al., 2022). The distribution of arsenic in different spheres of the environment is shown in Figure 1.1.

Table 1.1: General properties of arsenic

Element	Arsenic
Symbol	As
Atomic number	33
Atomic mass	74.921 u
Electronic configuration	[Ar] $3d^{10}4s^24p^3$
Boiling point	603°C
Melting point	817°C
Main allotropes	Yellow, grey and black (only the grey form has a metallic appearance)
Specific gravity	5.73 (grey form)
Solubility	As_2O_3 – 1.2 g at 0°C, 2.1 g at 25°C, 5.6 g at 75°C in 100 g of water As_2O_5 – 65.8 g in 100 g of water at 20°C
Isotopes	As^{60} to As^{92} (33 in number); As^{75} exists in stable form
Major ores	Arsenopyrite, realgar, arsenolite, enargite

1.4 Mobility of arsenic

Existence of arsenic in many different chemical forms makes its fate very complex. It is emitted in the atmosphere primarily in the form of As_2O_3 and arsines which further gets transformed to other species of arsenic and they ultimately get transported by winds and settle as dry and wet depositions (Frankenberger, 2002). With time, arsenic in air precipitates on soil which slowly gets mixed with groundwater and surface water through runoff (Nriagu et., 2007).

Arsenic in groundwater is regulated by oxidation-reduction potential and pH of groundwater, and oxidation state of arsenic (Hoang et al., 2010). Sorbed arsenic gets released in groundwater through reductive dissolution of hydrated iron oxide. Pyrite in volcanic zones can also be a source of arsenic in groundwater (Acharyya et al., 2005). When water level subsides, air enters the aquifer and oxidizes pyrite which contributes negligibly to arsenic pollution (Nickson et al., 2000).

Arsenopyrite (FeAsS) is the most abundant mineral which exists in anaerobic environments and also in many rock forming minerals, which contains arsenic (Smedley et al., 2002), and also occurs as a substitute of sulfur in the crystal lattice of many sulfide minerals. Iron and aluminium oxides present in sediments are the key players for contamination of groundwater (Shankar et al., 2014). When reduced by organic material, arsenic compounds from disintegrating rock can become soluble, leading to harmful concentrations of arsenic in both surface water and water from drilled wells. In well oxygenated waters, nearly all arsenic is present in arsenate form. So far, four mechanisms have been identified through which release of arsenic in groundwater takes place. The

mechanisms involve reductive dissolution and release of sorbed arsenic, oxidation of arsenic bearing sulfide minerals, desorption of arsenic and competitive adsorption/desorption reactions (Mukherjee et al., 2024).

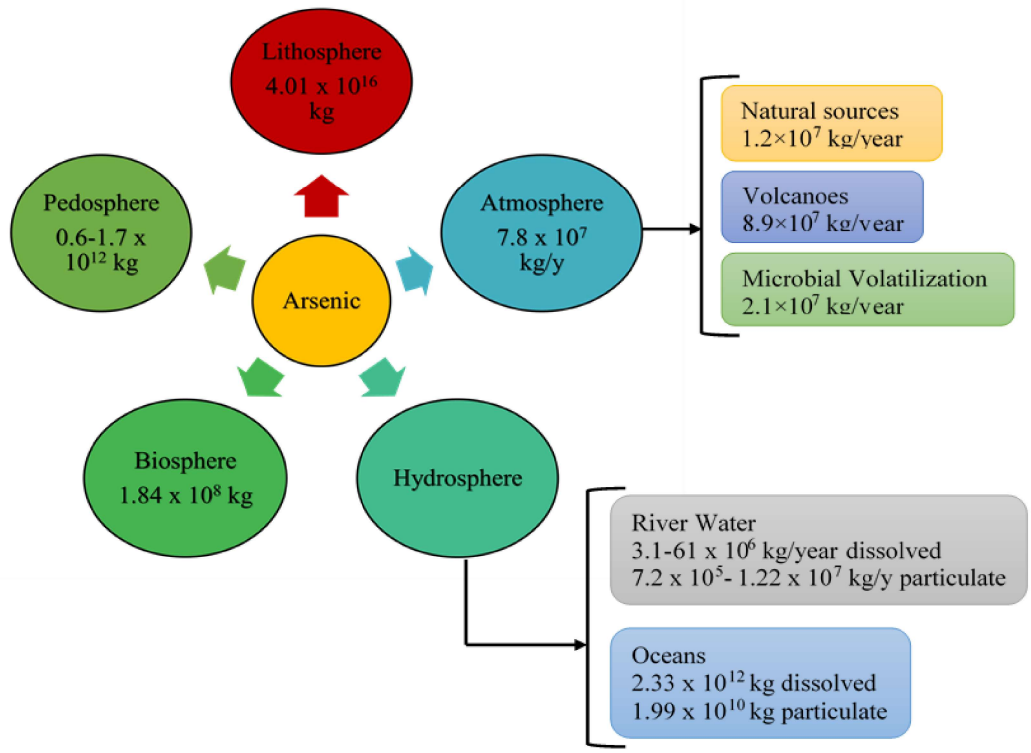

Figure 1.1: Distribution of arsenic in different spheres of the environment [adopted and modified from Masuda (2018)]

Microorganisms have also been found to play a crucial role in initiating the redox reactions that eventually control the mobility of arsenic. Studies suggest that in aerobic environment, negligible Fe(III) reduction and arsenic mobilization takes place with time; however, under anaerobic conditions, Fe(III) reduction and arsenic mobilization occurs simultaneously (Islam et al., 2004). The arsenic containing oxides of iron react with organic carbon and restrict the release of arsenic mediated by microbes (Stuckey et al., 2015).

In contaminated soils, the solubility and speciation of arsenic is influenced by pH in such a way that its mobility is higher in alkaline medium resulting in increased phytoavailability. Presence of other elements such as, aluminium, iron, manganese, etc. also affect the translocation of arsenic in different parts of the plant (Azam et al., 2016; Masscheleyn et al., 1991; Robles et al., 2016).

1.5 Health effects of arsenic

Contamination of arsenic is a problem worldwide (Shakya and Ghosh, 2019a,b; Singh et al., 2024) and it is among the top chemicals that pose a threat to human health as per the

World Health Organization (WHO); hence, the permissible limit in drinking water is not to increase beyond 10 µg/L (Sevak and Pushkar, 2024). Table 1.2 lists the permissible limit of arsenic in drinking water, industrial effluent, and surface water set by various agencies worldwide. The permissible limit in drinking water has not always been at 10 µg/L but has evolved over time through several amendments and revisions. Table 1.3 lists the evolution of permissible limit of arsenic in drinking water over time. Human exposure to arsenic can be through a number of ways, including drinking water, food, dust inhalation, etc. (Patel et al., 2023; Saha et al., 1999). As(III) is more mobile than As(V) and enters living beings more easily. As(III) enters the cell through aquaglyceroporins in yeasts, bacteria and mammals (Sevak and Pushkar, 2024). It binds with -SH (thio) groups in the amino acids of proteins of the cells and compromises the activity of cells by disrupting the enzymes (Achour et al., 2007).

The affinity of As(III) for thiols or vicinal sulfhydryl makes it highly toxic and impedes basic cell signaling by disrupting endocrine functioning (Tsai et al., 2009). As(V) uptake is facilitated by its structural similarity to inorganic phosphate and disrupts metabolic reactions that require phosphorylation (Achour et al., 2007), affecting metabolism and can also damage biomolecules. Arsenic is also responsible for the generation of reactive oxygen species (ROS), which adversely affects lipids, proteins and DNA, possibly resulting in cancer (Tsai et al., 2009).

Table 1.2: Permissible limit of arsenic set by various agencies in different water systems

Agency	**Drinking water**	**Surface water**	**Industrial effluent**
Bureau Indian Standards (BIS)	10 µg/L	50 µg/L	200 µg/L
United States Environmental Protection Agency (USEPA)	10 µg/L	10 µg/L	50 µg/L
World Health Organization (WHO)	10 µg/L	10 µg/L	100 µg/L

Arsenic has an extensive range of effects on human health. It has been known to cause epigenomic and epidemiological effects (Ozturk et al., 2022). Some of the health impacts of arsenic poisoning include its effects on the reproductive, kidney, cardiovascular, neurological and immune systems, and skin and hematological problems (Alka et al., 2021; Bundschuh et al., 2022). It has also been known to cause cell necrosis and deterioration of the gastrointestinal capillaries. Long-term contact with arsenic can also result in depression, anorexia, dehydration, unregulated body temperature, recurrent urination, etc. (Shrivastava et al., 2015). While there is no specific medicine for treating arsenic toxicity, consuming arsenic-free water can alleviate the symptoms (Adak et al., 2014).

Table 1.3: Evolution of permissible limit of arsenic in drinking water over time

Year	Standard/Specification	Permissible limit
	BIS	
1983	Drinking water - specifications	50 µg/L
1991	Drinking water - specifications	50 µg/L
2009	Drinking water - specifications	50 µg/L
2012	Drinking water - specifications	10 µg/L
	USEPA	
Before 2001	Standard for drinking water	50 µg/L
2001	Standard for drinking water	10 µg/L
	WHO	
1953	International standards for drinking water	200 µg/L
1963	International standards for drinking water	50 µg/L
1971	International standards for drinking water	50 µg/L
1984	Guidelines for drinking water quality	50 µg/L
1993	Guidelines for drinking water quality	10 µg/L

Apart from human health, arsenic also affects plants and animals. In plants, it is known to induce negative effects including delayed seed germination, stunted growth, lower yield, decreased chlorophyll content, withered and yellowing of leaves, etc. (Sevak and Pushkar, 2024). Arsenic interferes with plant metabolism, induces phytotoxicity and results in physiological, morphological and biochemical changes (Murugaiyan et al., 2019). In aquatic animals, it causes gill damage, bile stagnation, necrosis, poisoning, brain damage and cell death (Kumar et al., 2019). It also results in decreased hatchability and increased incubation period in fish eggs. High levels of arsenic are known to affect the microbial diversity negatively. It results in oxidative stress, leading to lower photosynthesis, and is also known to change metabolic pathways (Li et al., 2021; Tokmina-Lukaszewska et al., 2017). Figure 1.2 lists various effects of arsenic on human and environmental health.

1.6 Current state of arsenic contamination

1.6.1 Global scenario

Groundwater contamination of arsenic has been reported worldwide. Millions of people in over 100 countries across the world consume water contaminated with arsenic concentrations higher than the permissible limit (Dilpazeer et al., 2023; Shaji et al., 2021). Some of the worst affected countries include Bangladesh, Cambodia, China, Indonesia, India, Laos, Myanmar, Nepal, Pakistan, USA and Vietnam (Shaji et al., 2021). Other countries also affected by arsenic include Brazil, Egypt, Ethiopia, Ghana, Greece, Peru, Nigeria, etc. (Patel et al., 2023). Countries in South and Southeast Asia, such as Bangladesh, India and Nepal, are more severely affected than developed countries, such as the USA

(McArthur, 2019). In developing countries, agricultural and industrial activities are the main sources of arsenic contamination in surface and groundwater. In Bangladesh, 35 to 77 million people are estimated to be exposed to arsenic and approximately 43,000 deaths are reported yearly due to arsenic-related illnesses (Ivy et al., 2022). In Ghana, concentrations as high as 8,250 µg/L have been reported due to mining operations (Serfor-Armah et al., 2006). In North America, the concentrations can be as high as 3,000 µg/L (Smedley et al., 2002), whereas concentrations as high as 90,000 µg/L have been reported from different parts of Europe and 300,000 µg/L in Australia (Medunić et al., 2020). The level of arsenic contamination in some countries of various continents has been shown in Figure 1.3.

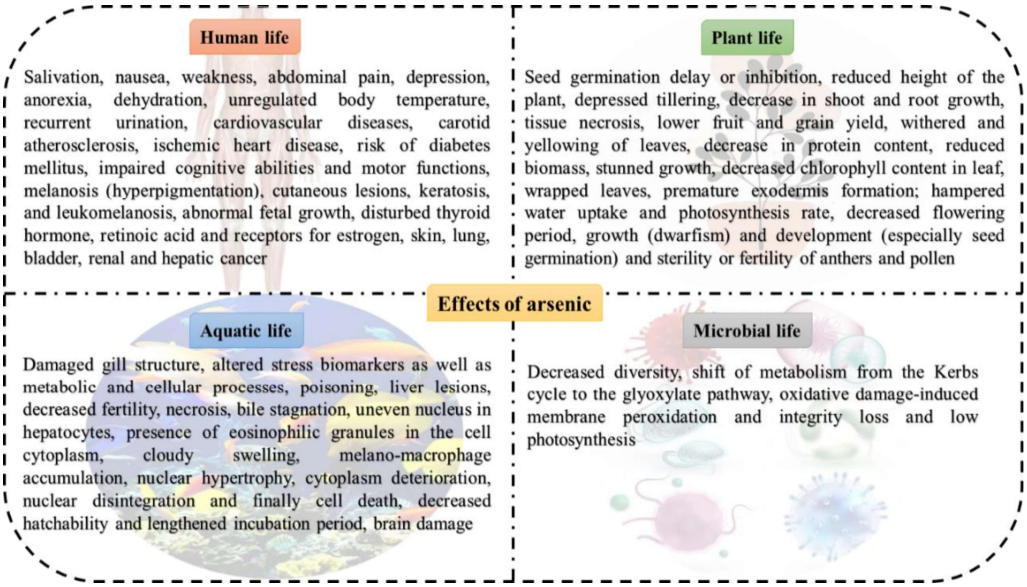

Figure 1.2: Effects of arsenic on human and environmental health

1.6.2 National scenario for India

In India, millions of people across 20 states and 4 union territories are at risk from arsenic-contaminated groundwater. The occurrence of arsenic in groundwater is mainly in the intermediate aquifers (20 – 100 m), whereas the deeper aquifers (>100 m) are free from arsenic (CGWB, 2015). In India, arsenic in groundwater occurs in (i) alluvial and (ii) hard rock terrane in high concentrations. Alluvial aquifers contribute 90% of arsenic, whereas hard rock aquifers, which include states like Karnataka and Chhattisgarh, account only for 10% (Shaji et al., 2021). The plains of Ganga in Uttar Pradesh, Bihar and Ganga-Brahmaputra-Meghna are the worst affected (Chakraborti et al., 2013). Concentrations of arsenic in groundwater vary significantly across the country. Maximum concentration of 569 µg/L in Assam, 1805 µg/L in Bihar, 985 µg/L in Chhattisgarh, 115 µg/L in Jharkhand, 235 µg/L in Karnataka, 1000 µg/L in Punjab, 620 µg/L in Tamil Nadu, 3192 µg/L in Uttar Pradesh and 4622 µg/L in West Bengal have been reported (Jha and Tripathi, 2021).

Contamination in soil is also very high in some parts of India. A maximum concentration of 4600 mg/kg has been reported in the rhizospheric soils of Ambagarh Tehsil of Chhattisgarh state (Patel et al., 2015). The level of arsenic contamination in various districts of some states has been shown in Figure 1.4. Uttar Pradesh is one of worst affected states in India. Around 40 districts in the state are contaminated with high concentration of arsenic (Mishra, 2019; Soni et al., 2024). Balia, Balrampur, Chaundauli, Faizabad, Gorakhpur, Lakhimpur Kheri, Pratapgarh, Siddharthnagar, Varanasi, etc. are amongst some of the districts suffering from problem of arsenic. As per Namrata et al. (2015), out of 40 districts surveyed in Uttar Pradesh, more than 20 districts had concentration above 50 µg/L. In Varanasi, villages such as Bahadurpur, Madhiya, Bhojpur, Ratanpur, Semra, Jalilpur, Kateswar, Bhakhara and Kodupur have arsenic concentration above permissible limit (Raju, 2012).

1.7 Technologies for arsenic removal

Various technologies are available for arsenic treatment, including physical, chemical and biological methods (Chaudhary and Dikshit, 2012). These methods include adsorption, bioremediation, chemical oxidation, coagulation-flocculation, co-precipitation, electrocoagulation, ion exchange, membrane technologies, solar oxidation, etc. They vary greatly in their applicability, efficiency, cost and complexity. Techniques such as coagulation, co-precipitation and sorption offer higher removal efficiency (~90%) but result in the generation of huge amounts of sludge.

Advanced techniques such as membrane technology and reverse osmosis have high efficiency (>95%) but are cost and energy-intensive and also result in high water loss. The operability and efficiency of these techniques also depend on various factors such as pH, presence of competing ions, initial concentrations, etc. (Katsoyiannis and Zouboulis, 2006). Since it is difficult to remove As(III) as compared to As(V), therefore, most of the technologies require pre-oxidation of As(III) to As(V) before remediation. There are also Do-It-Yourself techniques, such as solar oxidation and removal of arsenic (SORAS), Arsiron Nilogon, Sono-filter, etc. available for arsenic removal, which are easy to use and cost-effective. Table 1.4 lists the advantages and disadvantages of various technologies for arsenic removal from water. However, recently, the focus has been on the development of more cost-effective and environment-friendly techniques that can counter the limitations of existing conventional technologies.

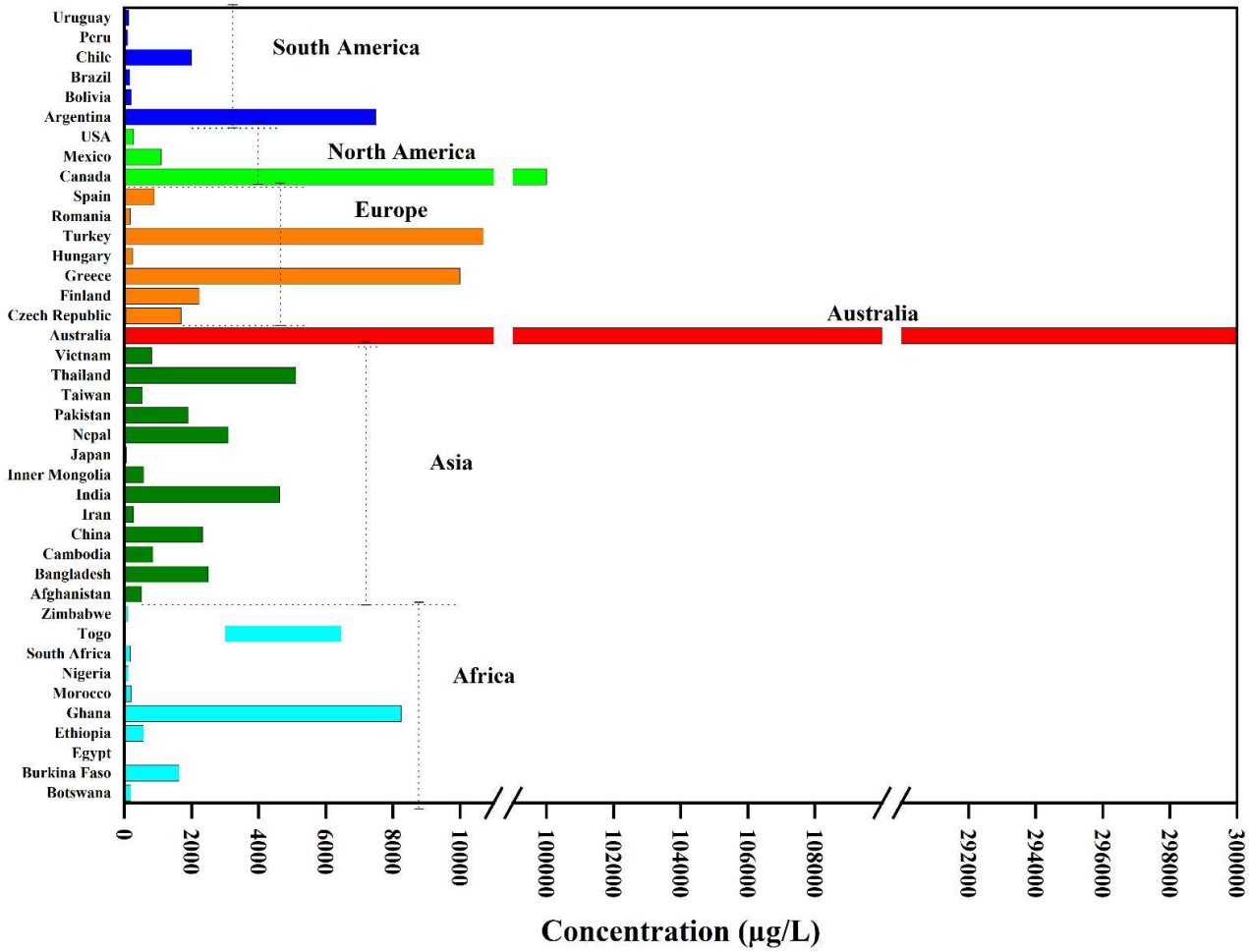

Figure 1.3: Concentration range of arsenic reported in various continents

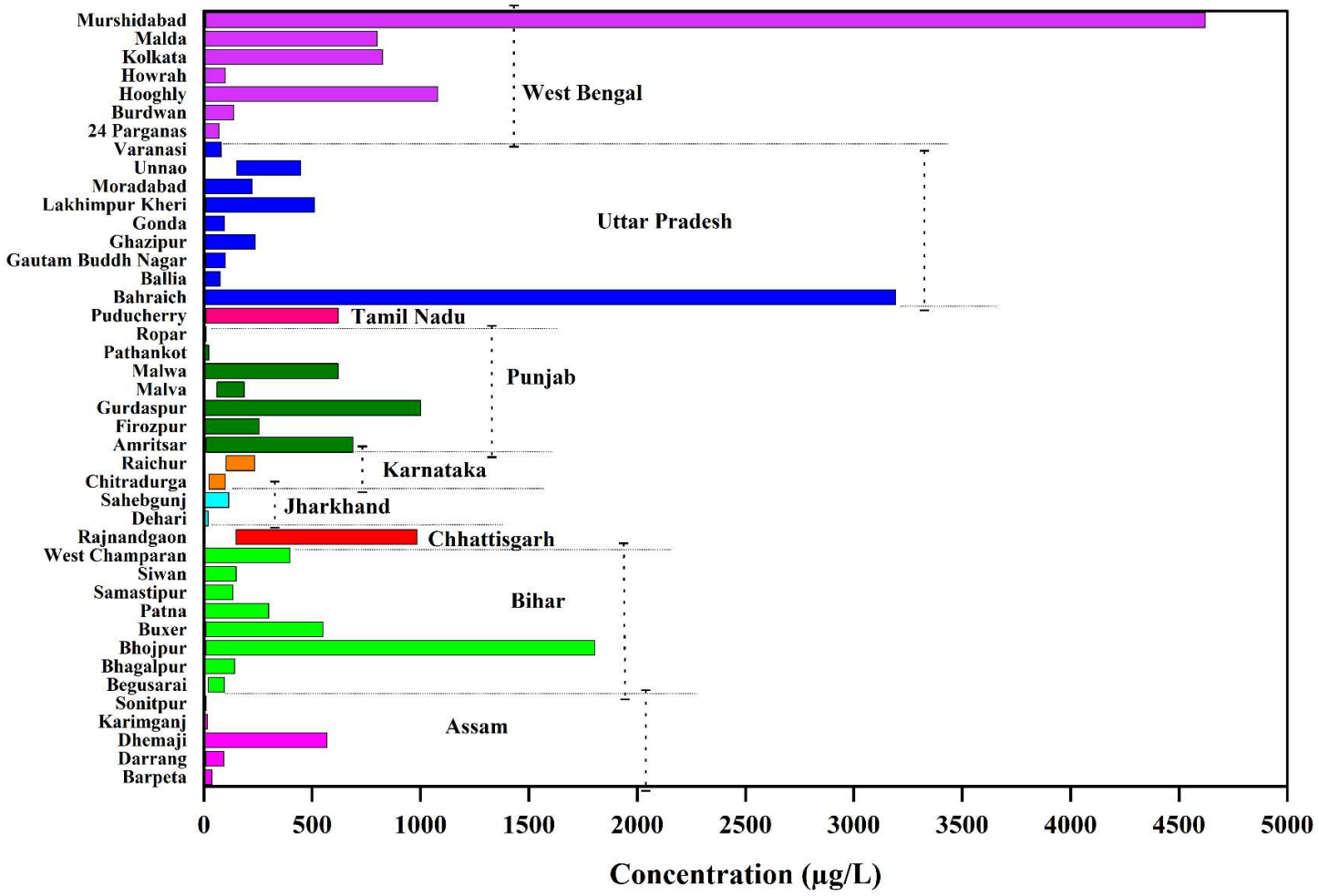

Figure 1.4: Concentration range of arsenic reported in certain states in India

Bioremediation has emerged as one of the most cost-effective and environment-friendly techniques of pollutant remediation (Geng et al., 2019). Using microorganisms or green plants to treat contaminants is one such method of arsenic removal. Microorganisms such as bacteria, algae, yeast and fungi are employed in bioremediation. Table 1.5 lists various approaches for bioremediation for arsenic. Apart from treatment technologies, various arsenic mitigation programs and measures such as household-scale and large-scale arsenic filtration and rural pipeline water supply installation have been initiated by different organizations like WHO and the United Nations. Adopting such technologies in remediation and mitigation programs still requires more investigation and participation from the management side of the organizations.

1.8 Co-contamination of arsenic and chromium

Chromium contamination is caused by both natural and anthropogenic sources (Mohan et al., 2005; Ghosh, 2009). It has been known to inhibit the growth of microorganisms at higher concentrations, inhibition of cytoskeleton and photosynthesis. In humans, it causes reproductive, respiratory, cardiovascular, mutagenic and genotoxic effects (Gupta et al., 2017; Sharma et al., 2022; Upadhyay and Sinha, 2021; Wang et al., 2014). India is severely affected with problem of chromium contamination. The maximum chromium concentration in all states of India have levels well above the permissible limit (0.05 mg/L) with Andhra Pradesh, Bihar, Maharashtra and Uttarakhand being the only exceptions (Poonia et al., 2021). In Uttar Pradesh, Kanpur, Kashi, Unnao, Varanasi and Vidyapeeth have problem of chromium contamination (CGWB, 2019). Kanpur Dehat has the highest concentration of chromium (33.5 mg/L) in the country (Poonia et al., 2021). In Varanasi, Sharma et al. (2007) reported chromium concentration between 0.03 to 0.09 mg/L in irrigation water whereas in wastewater irrigated soil, the concentration ranged between 13.40 and 679.89 mg/kg dry soil. Maurya et al. (2019) observed that in river water, chromium concentration was above the prescribed levels at all sampling locations. The maximum concentration in Varanasi in River Ganga was observed to be 0.45±0.06 mg/L.

Arsenic and chromium, both, are present in the earth's crust and their toxicity can occur simultaneously in soil due to several factors. Levels of arsenic can range from 1 to 20 mg/kg in natural and 45 to 2600 mg/kg in contaminated soils (Satapute et al., 2019), whereas the average concentration of chromium in the soil is 40 mg/kg (Zulfiqar et al., 2023). Industrial pollution, organic and inorganic fertilizers and wood preservatives such as chromated copper arsenate, which contain both arsenic and chromium, can result in extensive pollution of soil (de Oliveira et al., 2014).

Table 1.4: Brief description of various technologies available for arsenic removal

Basis of Technologies	Efficiency	Advantages	Disadvantages	Reference(s)
Oxidation/Precipitation • **Air oxidation** • **Chemical oxidation**	> 95%	**i.** Relatively simple, low-cost and rapid process **ii.** Also acts as disinfectant	**i.** Only part of arsenic is removed	Alka et al. (2021)
Coagulation • **Co-precipitation** • **Alum coagulation** • **Iron coagulation**	> 90%	**i.** Cost-effective and simple process **ii.** Easy availability of chemicals **iii.** Effective over a wide pH range	**i.** Sludge generation and disposal **ii.** May require pre-oxidation	Kameda et al. (2014)
Adsorption • **Activated carbon** • **Activated alumina** • **Iron coated sand** • **Biochar** • **Nanoparticles** • **Polymeric adsorbents**	> 95%	**i.** Commercially well established **ii.** Relatively simple technique **iii.** Low-cost, sludge free	**i.** Suitable for wastewater with low arsenic concentration **ii.** Needs replacement/ regeneration after a time period	Bulgariu et al. (2019); Donia et al. (2011); Mohan and Sonam (2018); (Tran et al. (2019); Tsai et al. (2022)
Ion Exchange	> 97%	**i.** Total removal and recuperation of metal **ii.** Limited sludge production	**i.** Periodic rejuvenation required **ii.** Cost intensive **iii.** Unfavorable order of selectivity	Alka et al. (2021)
Membrane Techniques • **Nano-filtration** • **Reverse osmosis** • **Electrodialysis**	96%	**i.** Highly efficient **ii.** No toxic byproduct **iii.** Other contaminants were also removed	**i.** Cost intensive **ii.** High technical operation and maintenance **iii.** High waste of water	Alka et al. (2021); Mohan and Sonam (2018)
Arsenic Removal Using Bottom Ash (ARUBA)	> 90%	**i.** Simple chemistry **ii.** High Efficiency	**i.** Not easily available **ii.** Requires separate manufacturing unit for substrate	Mathieu et al. (2010)

Bucket Treatment Unit developed by Department of Public Health Engineering (DPHE) -Danida	-	**i.** Useful for lower arsenic concentrations **ii.** Easy to use	**i.** Aluminum in treated water is harmful **ii.** Chances of bacterial contamination in the sand bed	Tahura et al. (1998)
SONO-Filter	> 95%	**i.** Removes other pollutants also **ii.** Easy to use	**i.** Failed to establish itself as a successful technology **ii.** Disposal of spent media	Hussam and Munir (2007); Kundu et al. (2016)
ArsIron Nilogon	> 99%	**i.** High efficiency **ii.** Simple, easy to use	**i.** Disposal of spent media	Bordoloi et al. (2015)
AMRIT – Arsenic and Metal Removal through Indian Technology	> 99%	**i.** Removes arsenic and other impurities as well **ii.** High efficiency	**i.** Disposal of spent media	Pradeep (2018)
Candle filter fitted with plastic bucket – AAIH&PH (All India Institute of Hygiene & Public Health)	80%	**i.** Simple, easy-to-use	**i.** Hazardous sludge **ii.** Not eco-friendly **iii.** Relatively lower efficiency	Majumder et al. (2013)
Amal filter	80%	**i.** Simple, easy-to-use	**i.** Residual aluminum **ii.** Not eco-friendly **iii.** Relatively lower efficiency	Majumder et al. (2013)
SORAS	> 90%	**i.** Simple, easy to use **ii.** Very low sludge generation **iii.** Does not require electricity or skilled labor	**i.** Requires more field tests	García et al. (2004); Mohan et al. (2022)

Table 1.5: Types of bioremediation technologies used for arsenic removal

Technologies	Efficiency	Advantages	Disadvantages	Reference(s)
Microbial • **Bacteria** • **Algae** • **Yeast and Fungi**	> 95%	**i.** High efficiency **ii.** Can transform As(III) to As(V) **iii.** Redox action can be used to construct microbial fuel cells (MFCs)	**i.** Transformation of As(V) to As(III) also occurs **ii.** The development of MFCs requires more research	Irshad et al. (2021)
Phytoremediation • **Phytostabilization** • **Phytoextraction** • **Phytodegradation** • **Phytovolatilization** • **Rhizofiltration**	99.9%	**i.** Land restoration **ii.** High economic value **iii.** Occurs naturally **iv.** High removal efficiency **v.** Prevents contaminant spreading	**i.** Lacks extensive field application **ii.** Time consuming **iii.** May produce additional toxic materials **iv.** Can negatively affect the growth of plants	Alka et al. (2021); Irshad et al. (2021)
Phytosuction Separation	-	**i.** Much more effective than phytoextraction **ii.** Occurs naturally **iii.** Other heavy metals also removed	**i.** Time-consuming, even for hyperaccumulators **ii.** Lacks extensive field application	Irshad et al. (2021)
Phytobial Bioremediation	99.9%	**i.** Bacteria enhance phytoremediation **ii.** Synergism between bacteria and plant	**i.** Slow and limited process	Shukla and Srivastava (2019)
Constructed Wetlands	> 95%	**i.** Low-cost and low-maintenance **ii.** Other heavy metals also removed **iii.** High removal efficiency	**i.** Leaching of bedding material may release arsenic **ii.** Difficult recovery and reuse	Irshad et al. (2021); Lizama-Allende et al. (2021)
Genetically Modified Organisms (GMOs)	-	**i.** Increases arsenic accumulation multiple times **ii.** Increased resistance to arsenic compared to wild species **iii.** Long-term effects and ethical concerns are also an issue	**i.** Survival of GMOs under natural conditions requires further research **ii.** Risk of adverse effects on human and environmental health	Irshad et al. (2021)

Chromium predominately exists as Cr(VI)/Cr(III) and arsenic as As(V)/As(III) with Cr(III) and As(V) being lesser toxic as compared to other valence states (Naz et al., 2020). Cr(VI) is about 300 times more toxic as compared to Cr(III) (Chen et al., 2021). Therefore, it becomes imperative to reduce Cr(VI) to Cr(III) state and oxidize As(III) to As(V) state for easy removal of these elements. Under such constraints, bioremediation is a promising option for removal of these co-contaminants together without putting any added burden to the environment.

1.9 Bioremediation

Bioremediation is the treatment of contaminants using biological means, namely microbes, plants, or their enzymes (Tabak et al., 2005). It may also involve a combination of microbes and plants. Various microbes such as bacteria (*Pseudomonas aeruginosa*, *Corynebacterium glutamicum*, *Acidithiobacillus thiooxidans*, etc.) (Sher and Rehman, 2019), algae (*Microcystis aeruginosa*, *Chlorella vulgaris*, etc.) (Jiang et al., 2011; Wang et al., 2013), fungi and yeast (*Aspergillus oryzae*, *Fusarium* sp., *Rhizomucor variabilis*, etc.) (Singh et al., 2015) and plants (*Armoracia rusticana*, *Lupinus albus*, *Helianthus annuus*, *Brassica juncea*, *Pteris vittate*, etc.) (Barbafieri et al., 2017; Kofroňová et al., 2019) have been used for treatment of various contaminants. Bioremediation can be broadly classified into two categories *in-situ* and *ex-situ* (Farhadian et al., 2008) (Figure 1.5).

***In-situ* bioremediation** – This technique involves treatment of contaminants on original site without a need to excavate or transport the contaminants to other sites. This method is generally preferred when it is not possible or is very costly to remove and transport large volumes of contaminated soil or water. *In-situ* remediation can be either intrinsic or engineered.

Intrinsic in-situ bioremediation, also known as natural reduction, involves treating polluted or contaminated sites without any external force (human intervention). This method depends on the microbial populations already in place at the contaminated site to break down or immobilize the pollutants (Sharma, 2020). The absence of human intervention implies that this method is much more cost-effective than other in-situ techniques.

Engineered in-situ bioremediation involves introduction of other microorganisms through human intervention at the place of contamination to improve bioremediation. Genetically modified organisms alter the physicochemical conditions to encourage the growth of microorganisms and enhance the degradation rate (Sharma, 2020). Engineered *in-situ* techniques can be further classified into bioaugmentation, biosparging, bioslurping, biostimulation, bioventing and natural attenuation, which have been defined in Table 1.6.

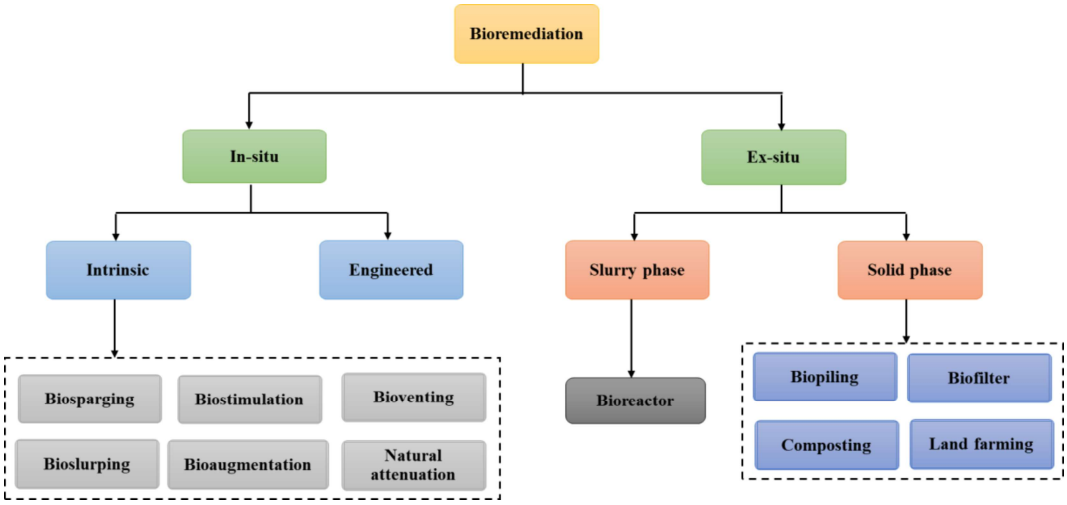

Figure 1.5: Classification of bioremediation processes

***Ex-situ* bioremediation** – It involves transporting pollutants to a different site for treatment. This method is chosen when *in-situ* treatment is not possible due to site constraints or when more precise quality control is required. *Ex-situ* treatment is broadly classified as solid phase and slurry *ex-situ* bioremediation.

Table 1.6: Engineered in-situ bioremediation techniques

Bioaugmentation	Involves the introduction of specific microbes or consortia to contaminated medium to enhance natural bioremediation.
Bioventing	It involves a controlled supply of air or oxygen to an unsaturated (vadose) zone to increase the activity of indigenous microbes to enhance bioremediation**.**
Bioslurping	It combines bioventing and vacuum-enhanced extraction for removal of contaminants from soil.
Biosparging	It is similar to bioventing, with the only difference being that air is supplied to the saturated zone.
Biostimulation	Creates conditions to support the growth and metabolic activity of microbes to enhance bioremediation.
Natural attenuation	It is also known as passive remediation and involves use of natural processes to degrade contaminants.

Solid phase ex-situ bioremediation is used to treat contaminated solid materials, soils, or sediments away from their original location. Organic wastes such as leaves, animal manures and agricultural wastes are included. Soil or sediment is placed into piles and bacterial growth simulated through a network of pipes are distributed throughout piles. Ventilation of microbes is provided by pulling air through pipes (Hyman and Dupont, 2001). Solid phase *ex-situ* bioremediation can be further classified into biofilter, biopiling, composting and land farming.

Biofilter is used to remove contaminants from gas or water streams. The contaminated stream is passed through the filter and pollutants are broken down by the microorganisms. A single organism or a consortium of organisms can be used for treatment.

Biopiling involves creating piles or heaps of contaminated soil or sediment, which is followed by the addition of nutrients and microbes to enhance the bioremediation rate.

Composting is the aerobic decomposition of organic materials using microbes under controlled conditions, resulting in a nuisance-free, sanitary, humus-like material that may be used as a soil conditioner.

Land farming entails covering a prepared land surface with contaminated soil and creating the right circumstances to promote microbial activity and the biodegradation of the contaminants. It is generally an *ex-situ* process; however, in some cases, it is also considered an *in-situ* process (Azubuike et al., 2016).

Slurry phase ex-situ bioremediation is a relatively faster mode of treatment as compared to solid phase treatment. Contaminated soil or sediment is mixed with water or other aqueous solutions to form a slurry, which is then subjected to controlled conditions to enhance microbial activity and promote the rate of biodegradation. Slurry phase bioremediation is performed in vessels commonly known as bioreactors. Bioreactors are designed for specific conditions; hence, it is essential to correctly design the reactor for optimum working conditions. Based on the operating modes, reactors can be classified as batch, completely mixed, fluidized bed, plug flow, packed bed, etc. (Table 1.7).

1.10 Factors affecting bioremediation process

The growth cycle of microbes consists of four stages: lag phase, exponential phase, stationary phase and death phase. The delay in the lag phase is due to the acclimatization of microbes with the substrate(s). After acclimatization, microbes enter the exponential growth phase, which is influenced by environmental conditions and characteristics of microbes. Once the entire nutrient is consumed, the exponential growth of microbes is inhibited and finally ceases and there is no net increase or decrease in microbial cells. Lastly, microbes enter the death phase and their viable number decreases. In the presence of proper conditions, all organic compounds can be degraded based on the growth cycle of microbes. However, the bioremediation rate is affected by various physical, chemical and biochemical conditions (Prasanna et al., 2008), some of which can be modified or controlled, whereas it is difficult for others.

Table 1.7: Types of reactors

Type of reactor	Description
Airlift	Airlift reactors are specialized reactors for biological cultures. It uses aeration to promote circulation. The culture is mixed and gassed by sparging air from the bottom of the reactor.
Arbitrary flow	This type of flow has a degree of mixing between plug flow and complete mix.
Batch	Fixed quantity of reactant is processed as a single batch and is mixed completely. Nothing goes in or out of the reactor.
Continuous-flow stirred tank, also known as complete-mix	It is used in the continuous processing of liquid reactants. Reactants are continuously fed into the reactor and products are continuously removed. The reactor is characterized by an agitator that ensures uniform mixing of the reactants throughout the entire reactor volume.
Fluidized flow	In a fluidized bed, solid particles such as support media or carrier materials are suspended by upward movement of fluid through the bed. The solid particles support microbial attachment in a bioreactor. The porosity of the packing can be varied by controlling the flow rate of the fluid.
Membrane	A membrane reactor combines a membrane-based separation unit within the reaction vessel. It integrates membrane separation and chemical reaction operations into one system. Membrane integration enables simultaneous reaction and separation, which increases yield, selectivity, and efficiency.
Moving bed	Moving bed reactor is a catalytic reactor in which a granular catalyst layer is moved between the reaction and regeneration area. The catalyst moves along with the reactants, separated at the exit and recycled after regeneration.
Packed bed	It consists of a column filled with a packing material that provides a surface for microbial attachment and growth, resulting in biofilm formation. Packed bed bioreactors can be either completely filled or intermittently dosed.
Plug-flow or tubular-flow	Fluid particles flow through a tubular system, with fluid entering at one end and leaving at the other. There is a high degree of axial mixing without any radial mixing. Particles remain in the tank for a time equal to theoretical detention time. These tanks have a high length-to-width ratio.

1.10.1 pH

Most natural environmental conditions exist between the pH range of 5.0 to 9.0. Therefore, it is the most optimum condition for bioremediation and most microbes exhibit optimum growth at circumneutral pH. Acidic pH (<3) and alkali pH (>10) significantly inhibit the growth of microbes (Juwarkar et al., 2010). Majority of microorganisms, apart from acidophiles and basophiles, are severely affected by extreme pH conditions. pH conditions

affect microbe metabolism, solubility of gases and metals, availability of nutrients. pH is maintained naturally by the buffering capacity of soils due to the presence of carbonates and other minerals.

1.10.2 Temperature

Biological activity doubles with every 10^{o}C increase in temperature. The biochemical rates increase with temperature up to a maximum and then decline due to the denaturation of enzymes, leading to decreased activity or even death of microbes (Trasar-Cepeda et al., 2007). Temperature affects metabolism, growth rate, soil matrix and state of contaminants. *In-situ* bioremediation is generally carried out in mesophilic conditions (20–45^{o}C) and hence, the majority of the laboratory studies are also focused on mesophilic microbes.

1.10.3 Metal(loid)s and toxic compounds

Metal(loid)s such as arsenic, copper, lead, mercury, etc., have toxic effects on microbes. The toxicity of metal(loid)s can inhibit the cellular processes of the microbes and their availability usually depends on pH, as metal(loid)s become more mobile at higher pH (Juwarkar et al., 2010). Microorganisms and plants actively accumulate metal(loid)s. Heavy metal(loid)s, although they may not be essential for the metabolism of microbes/plants, may accumulate in living cells and result in adverse effects. Many essential metal(loid)s, known as principal nutrients, such as iron, manganese, magnesium, etc., are required for the optimum growth of microbes/plants (Mishra et al., 2020). High concentrations of some compounds may be toxic to microbes and cause problems. These toxic compounds inhibit the growth of new cells. Compounds such as herbicides, pesticides, etc., are toxic to many micro and macro life forms.

1.10.4 Water content and geological characteristics

Water availability significantly influences the rate of bioremediation. Adequate moisture is essential for microbial growth and activity. However, excessive water can lead to anaerobic conditions, limiting the effectiveness of aerobic microorganisms. In the soil matrix, soil-water relationship is one of the most important parameters while assessing plant growth in bioremediated soils (Juwarkar et al., 2010). Generally, granular or porous soil having high permeability and uniform mineralogy results in enhanced *in-situ* bioremediation. On the other hand, low permeability, rocky conditions, complex mineralogy and water-logged or arid conditions inhibit microbial growth and are unfavorable for bioremediation (Bali et al., 2002).

1.10.5 Nutrient availability

Microorganisms require nutrients for their metabolic processes and its requirements depend on the nature of contaminants. Nutrients are generally supplemented in both *in-situ* and *ex-situ* methods of treatment and their availability, including nitrogen, phosphorus and trace elements, can influence the rate of bioremediation. Lack of essential nutrients can significantly affect microbial activity and inhibit their growth.

1.10.6 External electron availability

The availability of external electrons play a crucial role in influencing the rate of bioremediation. Some microbes use metal(loid)s as electron donors or acceptors to enhance bioremediation rates and the presence or absence of suitable electron donors or acceptors can significantly impact the overall efficiency of the bioremediation process. Despite its toxicity, various bacteria use arsenic either as an electron donor or an acceptor to fuel their metabolism (Kruger et al., 2013).

1.10.7 Bioavailability of pollutants

Interaction of contaminants with soils is complex, which impedes their availability to microorganisms and plants. Bacteria attaches itself to soil particles and hence, is constrained as a result. The chemical structure of contaminants can also affect their bioavailability. Some contaminants may require specific microbial processes for breakdown. Complex or recalcitrant compounds may require more energy for microbial degradation. Water solubility of contaminants also affects the bioremediation and dissolution of contaminants in the water phase is enhanced by adding surfactants.

1.10.8 Co-metabolism

Co-metabolism is a process in which microorganisms metabolize a secondary compound (co-substrate) apart from the main contaminant. Co-metabolism expands the range of contaminants that can be degraded by microorganisms. Also, the process may induce enzymes not directly involved in the bioremediation of primary contaminants and provide additional energy, resulting in an enhanced bioremediation rate.

1.10.9 Gene expression

Expression of genes in microorganisms is an important factor that affects the degradation of organic pollutants. The genes may not express if the substances are unavailable in sufficient quantities. This can be amended by adding substances similar to pollutants in structure and may act as inducers. However, the availability of alternate carbon sources may repress the enzyme expression needed for pollutant degradation. Gene expression plays a fundamental role in determining the rate and efficiency of bioremediation processes.

It is safe to deduce that bioremediation has emerged as an efficient, environment-friendly, safe and cost-effective technology for removal of contaminants without putting an additional burden on the environment (Mohan et al., 2006).

1.11 Organization of the Thesis

The Thesis has been divided into eight chapters, followed by references and appendices at the end.

Chapter 1: This chapter provides a background information with the goal to introduce the problem of arsenic, its effects on humans and environment, current scenario of arsenic contamination in world and in India. It also discusses about the technologies available for treatment of arsenic and briefly discusses about bioremediation and factors affecting the process of bioremediation.

Chapter 2: This chapter focuses on the review of literature on bioremediation of heavy elements in general and focuses especially on bioremediation of arsenic with different microorganisms and plants. It also elaborates about uptake and efflux pathways, genes involved in arsenic metabolism in bacteria, use of immobilized bacteria, encapsulation of bacteria and removal of arsenic from soil. The chapter concludes with research gaps and aims and objectives of the current research work.

Chapter 3: This chapter incorporates the materials and methodologies adopted in the studies. It discusses the different chemicals and media required, analytical techniques, the experimental setup and the methodologies implemented for different bioremediation and toxicity studies conducted.

Chapter 4: This chapter deals with the isolation and identification of arsenic resistant and remediating bacteria, various parameters affecting the bacterial growth, ability of bacteria to oxidize or reduce arsenic and genes involved and resistance of the isolated bacteria towards other heavy elements. Phytotoxicity study on chickpeas and *Vigna radiata* was also conducted.

Chapter 5: This chapter presents the studies conducted for bioremediation of arsenic in a recirculating packed bed bioreactor with immobilized bacteria. It also includes the comparative study with suspended and immobilized bacteria and bacterial toxicity assessment and bioluminescence inhibition.

Chapter 6: It consists of removal of arsenic and chromium(VI) from soil using bacteria. This chapter also includes comparison of different extraction agents for arsenic and chromium(VI), uptake of arsenic and chromium(VI) by chickpeas and chlorophyll assessment.

Chapter 7: This chapter discusses about removal of arsenic using biochar and bacteria immobilized on biochar encapsulated in polyvinyl alcohol (PVA) – sodium alginate (SA) beads. It also discusses about the isotherm and kinetic studies of biochar, its regeneration and disposal. Various parameters affecting the removal efficiency of beads, its water absorption capacity and removal of arsenic in recirculating packed bed bioreactor has also been discussed in this chapter.

Chapter 8: This chapter presents a summary of the current research work in the form of major conclusions drawn from the study, limitations of the present work and suggestions for further research.