Journal of Chemistry

Persistent Toxic Substances in the Environment and Humans 2021

Lead Guest Editor: Yifeng Zhang
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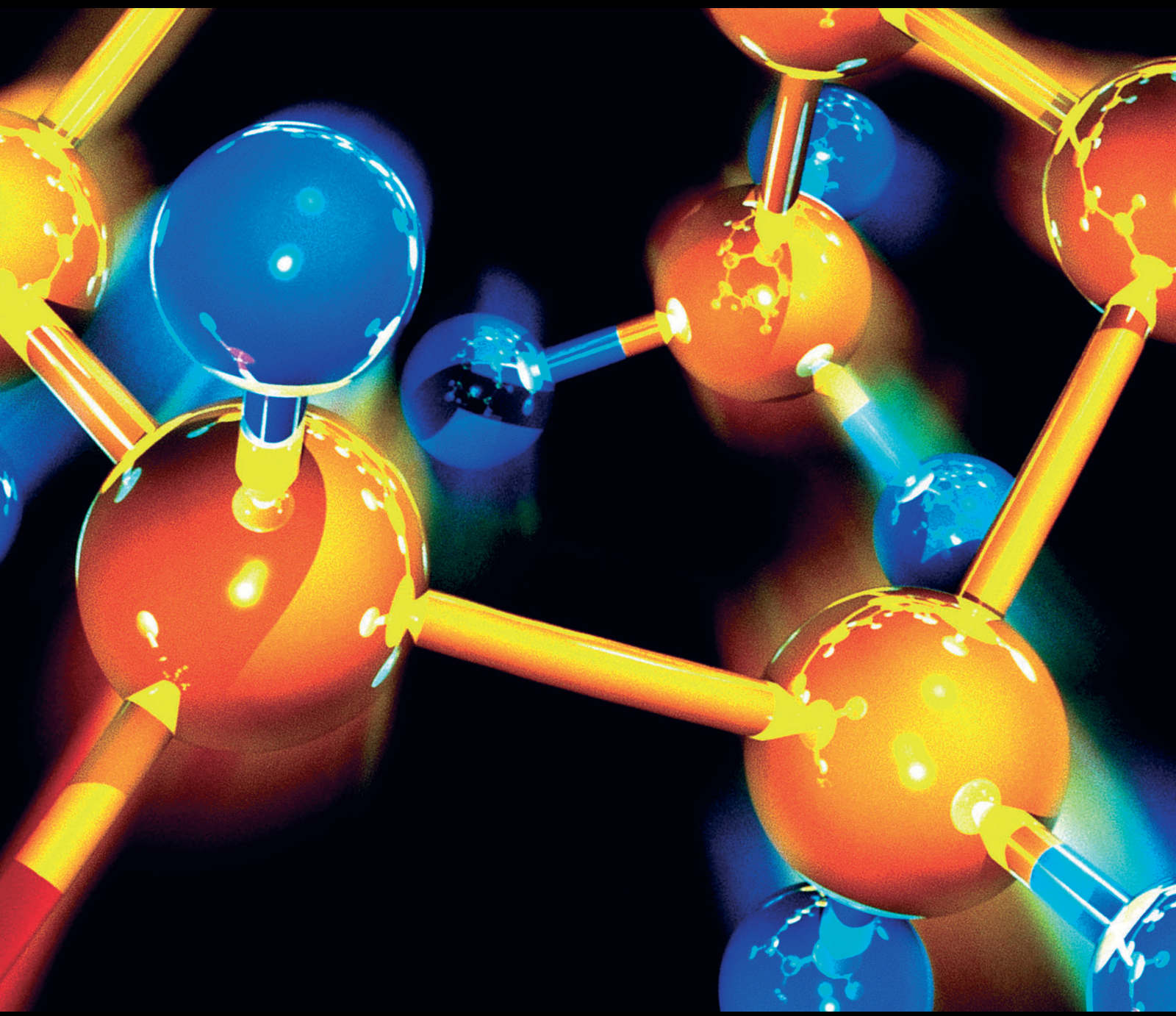

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Hindawi
Journal of Chemistry
Volume 2021, Article ID 9823362, 17 pages
https://doi.org/10.1155/2021/9823362

Review Article

Factors Influencing the Bacterial Bioremediation of Hydrocarbon Contaminants in the Soil: Mechanisms and Impacts

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Received 29 July 2021; Revised 26 September 2021; Accepted 27 October 2021; Published 15 November 2021

Academic Editor: Yuhe He

The discharge of hydrocarbons and their derivatives to environments due to human and/or natural activities cause environmental pollution (soil, water, and air) and affect the natural functioning of an ecosystem. To minimize or eradicate environmental pollution by hydrocarbon contaminants, studies showed strategies including physical, chemical, and biological approaches. Among those strategies, the use of biological techniques (especially bacterial biodegradation) is critically important to remove hydrocarbon contaminants. The current review discusses the insights of major factors that enhance or hinder the bacterial bioremediation of hydrocarbon contaminants (aliphatic, aromatic, and polyaromatic hydrocarbons) in the soil. The key factors limiting the overall hydrocarbon biodegradation are generally categorized as biotic factors and abiotic factors. Among various environmental factors, temperature range from 30 to 40°C, pH range from 5 to 8, moisture availability range from 30 to 90%, carbon/nitrogen/phosphorous (C/N/P; 100:20:1) ratio, and 10–40% of oxygen for aerobic degradation are the key factors that show positive correlation for greatest hydrocarbon biodegradation rate by altering the activities of the microbial and degradative enzymes in soil. In addition, the formation of biofilm and production of biosurfactants in hydrocarbon-polluted soil environments increase microbial adaptation to low bioavailability of hydrophobic compounds, and genes that encode for hydrocarbon degradative enzymes are critical for the potential of microbes to bioremediate soils contaminated with hydrocarbon pollutants. Therefore, this review works on the identification of factors for effective hydrocarbon biodegradation, understanding, and optimization of those factors that are essential and critical.

1. Introduction

The demand and utilization of petroleum-derived hydrocarbon products lead to the contamination of environments such as air, water, and soil. Unfortunately, the releasing of hydrocarbon contaminants into the soil naturally or anthropologically affects both biotic and abiotic components of ecosystems [1]. To overcome pollutions caused by various hydrocarbon-derived pollutants, many researchers have been showing that physical, chemical, and biological methods are routinely used for the treatment of contaminated sites [2, 3]. Moreover, biological remediation is a cost-effective, simple, efficient, noninvasive, apply in situ, ecologically friendly, sustainable, and applicable approach [4–7]. The bioremediation technique comprises phytoremediation (rhizodegradation, phytoextraction, phytovolatilization, phytodegradation, rhizofiltration, and rhizostabilization), mycoremediation, microbial remediation, and phycoremediation. Studies specified that microbial bioremediation (the use of microorganisms: bacteria, fungi, and microalgae) is the most efficient to transform pollutants into less toxic and less mobile form or to mineralize

hydrocarbon contaminants into H_2O, CO_2, and microbial biomass [8, 9]. Among various microorganisms, bacteria are the most active and the primary hydrocarbon degraders [10]. Microorganisms that can survive and proliferate on hydrocarbon pollutants as their sole source of carbon and energy have evolved due to the introduction of hydrocarbon pollutants into the environment. Increased metabolic capacities could be gained through horizontal/vertical gene transfer and mutations, thereby acquiring genes whose products are involved in the breakdown of hydrocarbons. Even though such microbes have the potential to degrade hydrocarbon contaminants, certain limiting factors (biotic and abiotic) impinge on the efficient biodegradation of contaminants [11, 12]. Hydrocarbon and its associated environmental problems are received much attention in recent years worldwide. Consequently, there is noteworthy attention to degrade or eliminate pollutants using the natural population of microbes (bacteria, fungi, and microalgae) to have a pollution-free environment. For this, several studies have reported the impacts of various hydrocarbon and diverse remediation techniques. Based on historical publications, bibliometric analysis is a popular and successful tool for determining research trends and relevant concerns. It has been employed in a variety of scientific and engineering fields [13]. The keywords are provided in the Scopus database as follows: "TITLE-ABS-KEY ({bioremediation} AND {hydrocarbon} AND {soil} AND {factors}," to find the critical evaluation of the articles that discussed major optimization factors in bioremediation of hydrocarbons in soil from 2012–2022. The database showed 417 articles, and their year-wise publication is depicted in Figure 1(a). The country-wise search showed that the United States held the major number of publications (Figure 2(b)), and the type of document search revealed that a high number of publications are published under the article category (Figure 1(c)). The major three subject areas covered in the query is falling under environment science, immunology and microbiology, and agricultural and biological science (Figure 1(d)). The numerical values of articles based on the provided keywords in the reputed database collection such as "Scopus" indicate the continuous research development in the concerned field, which highly promotes further research and review. The application of bioremediation technology is not supreme, and it is affected by numerous factors that hamper its practical application, limiting the large-scale application of the techniques for hydrocarbon biodegradation practices. Accordingly, bacterial bioremediation of hydrocarbons in various environmental systems has received all-inclusive interest, and a systematic review is most needed to highlight the factors affecting the rate of bacterial bioremediation. Hence, the current review is designed to elucidate the key factors that influence the bacterial bioremediation of hydrocarbon in soil with their impacts.

2. Hydrocarbon Contaminations and Their Sources

Hydrocarbons are naturally occurring compounds that are principally formed from plant and animal fossils due to natural or anthropogenic actions. They are made up entirely of carbon and hydrogen atoms and serve as the foundation for crude oil, natural gas, and coal, providing a significant proportion of the world's energy. Hydrocarbons are classified as saturates (branched and straight-chained), aromatic (mono and polycyclic), resins (pyridines, quinolines, carbazoles, sulfoxides, and amides), and asphaltenes (phenols, fatty acids, ketones, esters, and porphyrins) [8, 14, 15]. Hydrocarbons and their derivatives were intentionally or accidentally discharged into the environment, mainly to the soil and oceans, and principally existed in three physical states, that is, solid, liquid, or gaseous [16]. The major sources of contamination are human activities (anthropogenic) and natural processes. The anthropogenic activities are related to the current increase in industrialization and civilization and generally contribute to the increased accumulation of hydrocarbon contaminants in the environment. Thus, sources include garages, gas station services, mining, wastes from chemical and petrochemical industries, agriculture wastes, incomplete combustion of organic matter, sludge wastes, gasification, automobile exhaust and processing, production, transportation, run-off asphalt pavements, volcanic eruption, the distillation of wood, waste disposals, vehicular emission, and combustion of fossil fuel [14, 17, 18]. Thereby, most hydrocarbon-contaminated environments contain light petroleum products (oil, gasoline, kerosene, and diesel), heavy hydrocarbons (environments lubricants, heavy oil, and crude oil), halogenated solvents, and other more complex molecules (aromatic hydrocarbons) [1, 11]. The difficulty of cleaning up of environment contaminated with hydrocarbon pollutants is the ability to identify potential sources either point or nonpoint sources. Consequently, differentiating and identifying of origin of hydrocarbon contaminants help apply imperative bioremediation techniques.

3. Environmental and Health Impact of Hydrocarbon Contamination

Pollution from hydrocarbon is a worldwide exemplar that raises animal, environmental, and human health concerns. Recently, the potential of hydrocarbon contaminants has received increasing attention of particular concern on the aquatic, marine, and land environment. Studies have been indicated that hydrocarbon can have a significant adverse impact on ecosystems [19]. Thereby, hydrocarbon contamination leads to the deterioration of the ecosystem's functioning and its living (fauna) and nonliving components [20]. In addition, when hydrocarbons are introduced into the soil, they can hamper the supply of water, nutrients, oxygen, light, and other parameters for the biological processes. This can affect soil fertility (plant growth and seed germination) and consequently agricultural productivity [17, 21]. Hydrocarbon contaminants cause immediate or latent effects such as genetic mutations, immunotoxicity, teratogenicity, carcinogenesis, high bioaccumulation potential, and deterioration of the ecosystem functioning and treating of animal and plant life [1, 14, 22–26]. The xenobiotic form of hydrocarbons can also sorb to organic-rich soils and sediments, accumulate in organisms (fish, plants, and other aquatic organisms), and may be transferred to the

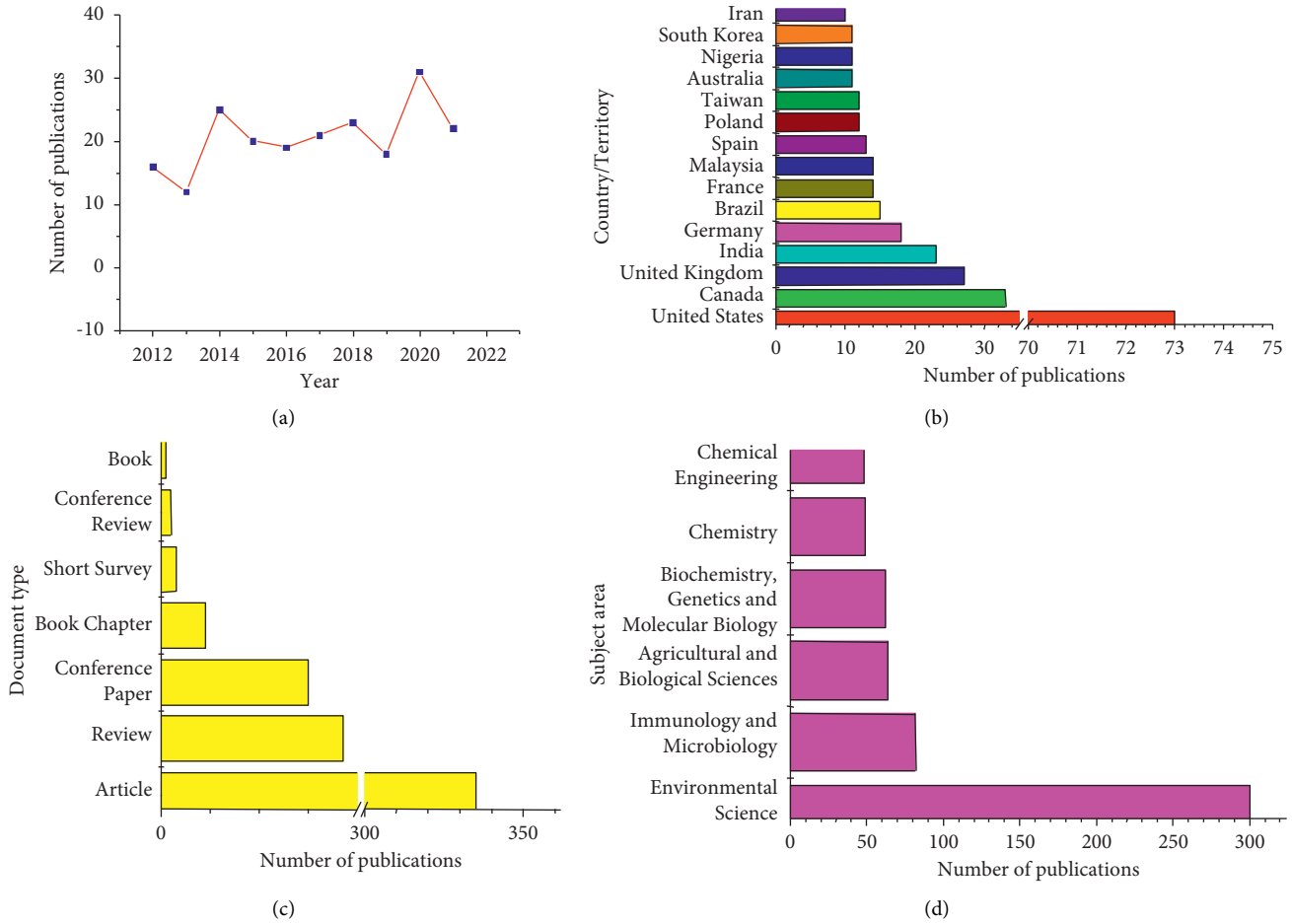

Figure 1: Graphical representation for the scopus database document output for the keywords (TITLE-ABS-KEY ({bioremediation} AND {hydrocarbon} AND {soil} AND {factors}) (Scopus database as on 16 July 2021): (a) documents for year-wise for the period of 2012–2021, (b) documents by major fifteen countries/territories, (c) documents by type, and (d) documents by major six subject areas.

food chain and badly disturb the exposed organisms [17]. The bioaccumulation and biomagnification of hydrocarbons also cause defects in the reproductive, immune system, neurologic, and developmental stages (teratogenic) and also cause cancers of the skin, lung, bladder, liver, and gastrointestinal [9, 23]. Therefore, exposure to hydrocarbon contaminants causes several health impacts. In addition, the hydrocarbon contamination and its toxicity diminish species richness, evenness, and phylogenetic variety, with the resulting community in a contaminated soil environment [27]. Therefore, hydrocarbon contaminants in the environment are known to cause significant impairment to the environment and organisms. Due to this and other complex problems with contamination of hydrocarbon components, microorganisms are isolated and screened as environmental remediation agents for increasing the rate of removal of the contaminants to make a healthy environment.

4. Hydrocarbon Bioremediation in the Soil

Bioremediation refers to the removal, destruction, immobilization, mineralization, or transformation of contaminants from the soil with the use of plants, protozoa, fungi, and microalgae [28, 29] and particularly bacteria [3] and their products in the presence of optimal conditions. Bioremediation methods with low operational costs for the removal or reduction of inorganic and organic pollutants (hydrocarbons, halogenated organic compounds, halogenated organic solvent, nonchlorinated pesticides and herbicides, nitrogen compounds, and heavy metals (lead, mercury, chromium, radionuclide, etc.) at least to the level that they cannot cause a serious effect to ecological functions [29, 30]. For such application, various macro- and microorganisms are used, but bacteria are selectively useful to degrade a variety of hydrocarbon pollutants and are ubiquitous [29, 31] and applied for in situ or ex situ bioremediation processes. In situ bioremediation refers to treating contaminants at the site with minimal disturbance using methods such as bioventing, biostimulation, bioaugmentation, and/or natural attenuation [32], while ex situ refers to the destruction or treatment of the contaminant from the site by using methods such as excavation, landfilling, composting, incineration, biopiles, and bioreactors [33]. In general, the different methods of bioremediation mechanisms are broadly categorized into natural attenuation, bioaugmentation, and biostimulation [20] (Figure 2 and Table 1).

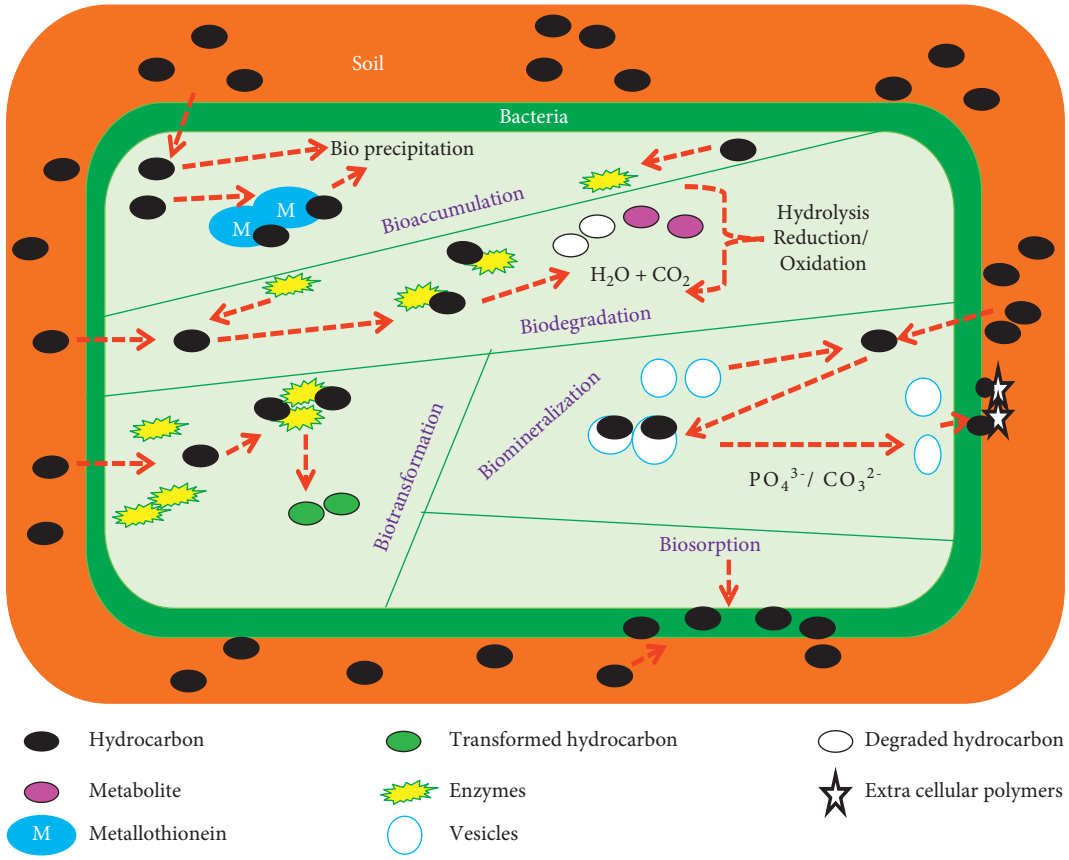

Figure 2: The mechanisms of microbial remediation used for reducing heavy metals [34].

4.1. Natural Attenuation/Intrinsic Bioremediation. Natural attenuation (natural remediation) refers to the use of indigenous microbial populations to eliminate or detoxify hazardous hydrocarbon pollutants into less or nontoxic forms [47]. The time required essential for natural attenuation depends on contamination, site conditions, and applicability of potential degrading bacteria. This implies that the use of natural attenuation is cost-effective and efficient if there is no need for a complex remediation process [47]. Thus, researches showed that this strategy is effective in degrading 25% of hydrocarbon pollutants in soil [15]. During this process, the indigenous microbes utilize hydrocarbon contaminants as the sole carbon and energy sources based on their natural metabolic pathways [48]. When the soil is contaminated with hydrocarbon contaminants, the number of indigenous hydrocarbon-degrading microorganisms increase rapidly and start to adapt to and metabolize (degrade) the pollutants. The bacterial metabolic pathways for the degradation of hydrocarbon contaminants are illustrated in Figure 3. Studies showed that natural attenuation is dependent on naturally occurring (indigenous) hydrocarbon degraders [9]. However, its effectiveness is contingent on nutrient availability, type and concentration of contaminants, physical parameters, the fate of contaminants, and potential microbial communities with necessary catabolic genes for complete hydrocarbon degradation. Albeit, natural attenuation is often a slow process, and its effectiveness can be achieved when supported with bioaugmentation and biostimulation techniques [21, 25].

4.2. Bioaugmentation. Bioaugmentation refers to the introduction of potential microbial strain or consortia isolated from other contaminated sites or the genetically modified microbes to support the remediation [49, 50]. This strategy is usually applied when natural attenuation is ineffective due to low indigenous hydrocarbon-degrading population, sluggish decontamination activities, and high-stress situations to start the bioremediation process [10]. For successful hydrocarbon biodegradation, the inocula must be able to metabolize broad ranges contaminants, not undergo mutation, withstand various biotic and abiotic factors, and effectively compete with other microorganisms (indigenous hydrocarbon-degrading and predators) and can easily reach contaminants in deep via pores of the sediment [51]. For the bioremediation approaches, microbial cell bioaugmentation is a commonly used technique for the removal of contaminants. In addition, there are several bioaugmentation techniques: phytoaugmentation, rhizosphere bioaugmentation, gene bioaugmentation that have been exercised for cleaning up contaminated sites.

4.3. Biostimulation. Biostimulation is the addition of growth-limiting factors such as substrates, vitamins, oxygen, and modifying the environmental conditions (temperature, moisture, pH, redox potential, terminal electron acceptors, etc.) in the hydrocarbon-contaminated environments [49, 50, 52]. This is important to improve the metabolic

Table 1: Overview of strategies and mechanisms employed for bioremediation of hydrocarbon.

Contaminant	Mode of test	Removal mechanism	Duration	Efficiency	Reference
Crude oil (TPH at 20 g/kg)	Soil microcosms	Bioaugmentation + biostimulation	30 days	36–51%	[35]
Crude oil (either 20 g/kg or 50 g/kg)	Field study in soil	Biostimulation + surfactant-assisted biodegradation	486 days	49–62%	[36]
Crude oil (20 g/kg)	Bioreactors with soil	Natural attenuation + bioaugmentation + biostimulation	60 days	51–90%	[37]
PAHs (574 mg/kg)	Soil microcosms	Surfactant-assisted biodegradation	84 days	72–77%	[38]
PAHs: Phenanthrene, fluoranthene, and pyrene (6 mg/kg)	Soil microcosms	Surfactant-assisted biodegradation	Up to 35 days	72% for phenanthrene, 64% for fluoranthene, and 58% for pyrene at day 7	[39]
PAHs (1.5 g/kg)	Soil mesocosms	Bioaugmentation + biostimulation	56 days	99%	[40]
Diesel oil and diesel/biodiesel blends (1% v/w)	Soil microcosms	Bioaugmentation	64.5 weeks	88–97%	[41]
Pyrene (10 mg/kg)	Soil microcosms	Bioaugmentation + biosurfactant/surfactant-assisted biodegradation	10 days	60%	[42]
Diesel oil hydrocarbons (3 g/kg) + PAHs (400 μg/kg)	Weathered oily soil biopiles	Bioaugmentation + biostimulation + surfactant-assisted biodegradation	160 days	39% for diesel oil hydrocarbons and 32% for PAHs	[43]
Diesel oil (1% v/v)	Flask tests	Natural attenuation + autochthonous bioaugmentation	7 days	20–40%	[44]
Engine oil (39–41 g/kg TPH)	Soil microcosms	Natural attenuation + bioaugmentation + biostimulation	210 days	31–75%	[45]
Petroleum refinery waste (TPH at 144 g/kg)	Vial microcosms	Natural attenuation + bioaugmentation + biostimulation	120 days	57–75%	[46]

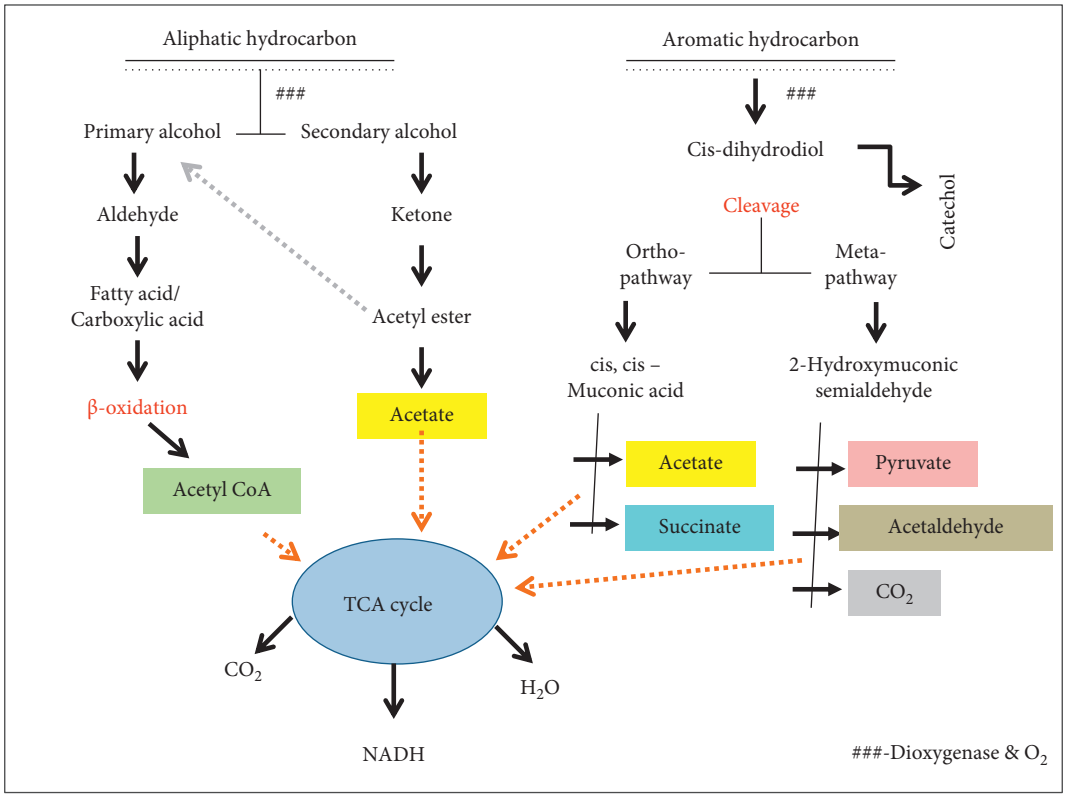

Figure 3: Bacterial metabolic pathways for the degradation of hydrocarbon contaminants [30].

capability of autochthonous hydrocarbon-degrading bacteria. When hydrocarbon contaminants are greatly available, they provide high carbon and energy but nitrogen or phosphorus for bacterial growth [31]. Hence, there is the need for the addition of nutrients in hydrocarbon-contaminated sites to increase the required nutrients and stimulate the growth of indigenous bacteria [52]. However, there are certain limitations for effective biostimulation application. These include the rapid depletion of major inorganic nutrients (NP), the nature of the soil, and the scarcity of indigenous hydrocarbon-degrading bacteria [53]. When indigenous or autochthonous microbial species are not fast enough to degrade pollution, microbial activity can be stimulated with the supplement of nutrients in the form of fertilizers such as KNO_3, $NaNO_3$, NH_3NO_3, K_2HPO_4, and $MgNH_4PO_4$ [47]. Henceforward, if there are hydrocarbon-degrading bacteria in the hydrocarbon polluted environments, it requires the supply of nutrients (biostimulation) and oxygen (via bioventing or biosparging), for rapid degradation of hydrocarbon contaminants [54]. Therefore, biostimulation is important to increase the efficiency of hydrocarbon-degrading bacteria. In general, the simultaneous application of bioaugmentation and biostimulation brings substantial and operative hydrocarbon bioremediation approaches by considering the major factors affecting the complete degradation of contaminants in the environment [21]. These factors are discussed below in detail.

5. Factors Affecting Bacterial Bioremediation

Many kinds of research have been conducted on the bioremediation of hydrocarbon-contaminated soil. Researchers have been proved that the use of a single strain or consortia of indigenous bacteria in the laboratory is effective for the biodegradation of hydrocarbon but often limited in the field [23]. This is because of the prevalence of diverse factors that affect the efficiency and rate of biodegradation. The overview of major classes of factors influencing the bacterial remediation of hydrocarbons in the soil is summarized in Figure 4. The major factors include characteristics of microorganisms (microbial consortia, metabolic potential, population density, ability to produce biosurfactant, and competition), the physicochemical properties of contaminants (chemical structure, concentration, toxicity, and bioavailability), and environmental factors (soil type, temperature, pH, oxygen, salinity, nutrient, and water availability) [24, 33, 55, 56]. Those factors alter microbial activities, degradative enzyme activities, and hydrocarbon degradation in general. This is an indication that bacterial bioremediation could be effective and enhanced if these factors are manipulation, optimized and regulated.

6. Biotic Factors

6.1. The Availability of Hydrocarbon-Degrading Bacteria. Hydrocarbon degrading bacteria are abundantly and ubiquitously found in hydrocarbon-contaminated soils. This is because those bacteria can easily adapt to the hydrocarbon-contaminated sites and use the contaminant as a source of carbon and energy for their metabolism and growth. The rate of hydrocarbon biodegradation is directly correlated to the availability of naturally existing potential hydrocarbon-degrading organisms in the contaminated environment [57]. Those potential bacteria are metabolically active and can degrade hydrocarbon contaminants aerobically or anaerobically. However, their availability in terms of species abundance and richness is closely related to the types and nature of hydrocarbon contaminants and the surrounding environmental factors [27]. Hence, the availability of naturally occurring suitable bacteria is crucial for the implementation of microbial remedial actions.

6.2. Bacterial Competition and Cooperation. Bacterial cooperation and competition is vital force for survival and stability within microbial communities of a given ecosystem [58]. Hydrocarbon degrading bacterial communities exist in cooperation (with synergistic effect) and/or competition (with antagonistic effect) for their survival in hydrocarbon-contaminated environments [59]. However, the bacterial competition of either interspecific (between bacteria and fungi) or intraspecific (between bacteria species themselves) is a limiting factor for biodegradation efficacy. For instance, for interspecific competition: hydrocarbon-degrading fungi and hydrocarbon metabolizing bacteria can compete to utilize hydrocarbon contaminants as carbon sources and other limited nutrients available (nitrogen and phosphorous) for their growth and metabolism. In addition, some hydrocarbon-degrading microbial species also release metabolites that inhibit the growth and development of other hydrocarbon-degrading bacterial species [49]. Studies showed that exogenous bacteria (introduced inocula) are used to degrade hydrocarbon contaminants [20] but are not usually effective. This is because they are unable to avoid competition with indigenous bacteria, predators, and various abiotic factors [53]. The study confirmed that the synergistic association between *Cycloclasticus* and *Alcanivorax borkumensis* enhances the degradation of polyaromatic hydrocarbons and antagonistic effect between *Thalassolituus* and other oleophobic bacteria leading to lessen the rate of biodegradation [59]. It was indicated that *A. borkumensis* produces surface-active substances (biosurfactant) to increase the emulsification of PAHs to be readily accessible for *Cycloclasticus* and no competition for PAHs for carbon source since *A. borkumensis* uses other hydrocarbon contaminants as a metabolite. However, *Thalassolituus* produces metabolites that can antagonize other oleophilic bacterial growth and development. The study also showed that the coexistence or interaction between indigenous and exogenous microbes antagonistically obstructs the metabolic activities of each other and thereby limits the effectiveness of hydrocarbon biodegradation [9]. Therefore, the interdependence of microbial populations (synergistic effect) is important for the successful application of bioremediation. It has been shown that some hydrocarbon-degrading microbial consortia develop synergistic relationships for complete degradation [60]. Thus, the bacterial consortia (community) provide comprehensive perceptions

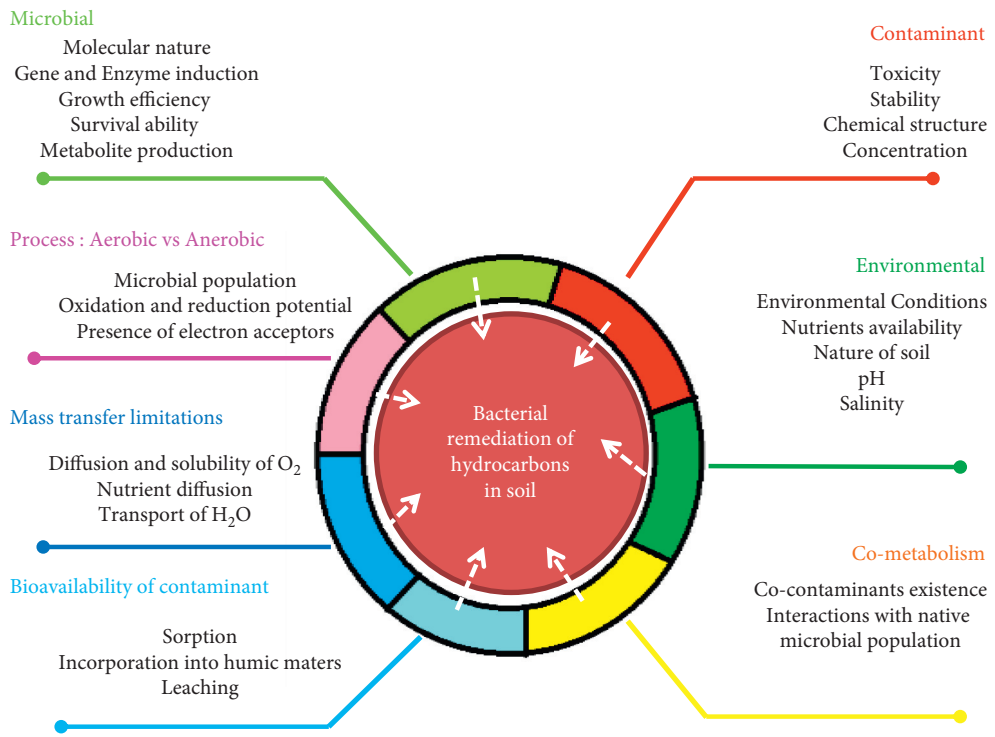

Figure 4: Major classes of factors influencing the bacterial remediation of hydrocarbons in soil.

about the modulation of their potential in hydrocarbon biodegradation and can provide useful information for in situ bioremediation of hydrocarbon-related pollutions.

6.3. Indigenous and Exogenous Hydrocarbon-Degrading Bacteria. Naturally occurring or introduced microbes (single or microbial communities) may be involved in removing hydrocarbon contaminants. However, native bacterial populations (indigenous and autochthonous) are competent than introduced inocula (exogenous and allochthonous) to mineralize hydrocarbon pollutants for the long-term success of the bioremediation process [61]. The microorganism that possesses a high degree of stability and physiological adaptations (specific catabolic actions) suited to local nutrient availability and environmental conditions is important to degrade various hydrocarbon components from contaminated sites [30]. Indigenous bacteria showed more efficient biodegradation at the rate of about as compared to exogenous consortia [53]. This is because exogenous bacteria are unable to withstand abiotic factors stress and are firm to live and propagate in the soil environment [58]. As a result, using indigenous inocula of specific bacterial strains or microbial consortia isolated from hydrocarbon-contaminated environments acclimates well to the local environment and is suggested for efficient and inexpensive bacterial bioremediation.

6.4. Bacterial Community Structure and Diversity/Microbial Consortia. To develop bioremediation techniques or strategies for hydrocarbon degradation, there is the need to have a better understanding of the dynamics of the bacterial community [62]. The soil environments contaminated with hydrocarbon pollutants change microbial community structure (diversity, richness, and evenness), thereby affecting the metabolic activity of hydrocarbon-degrading bacteria [63]. Studies also showed that when hydrocarbon contaminants are introduced to the soil environment, bacterial communities shift and can show instant and significant reductions in some bacterial species, while others became favorably more plentiful [60]. Supporting this, in long hydrocarbon-contaminated sites, the distribution and abundance of microbial communities were found to be in the increasing order of Chlamydiae, Elusimicrobia, Thermi, Cyanobacteria, Verrucomicrobia, Chloroflexi, Acidobacteria, Gemmatimonadetes, Firmicutes, Actinobacteria, Bacteroidetes, and Proteobacteria [5]. Thus, the hydrocarbon-degrading bacterial community structure and diversity showed that bacterial consortium (indigenous or introduced) is efficient than a single strain for complete degradation of hydrocarbon contaminants in the soil [10, 27]. This is for the reason that a single bacterial isolate (monoculture) can only utilize a limited range of hydrocarbon substrates [64], while mixed bacterial isolates (polyculture and consortium) are efficient to metabolize highly concentrated and broad hydrocarbon contaminants [30]. This is because consortia of bacteria possess broad degradative enzymes, degrade different hydrocarbon components (aliphatic and aromatics), have a higher tolerance to physic-chemical parameters (temperature, pH, and salinity) and a higher rate of surface-active biomolecules (biosurfactants) synthesis, and cope up with synergistic effect to maximize the rate of bioremediation [65, 66].

Therefore, understanding community structure, diversity, and interaction is essential to define the metabolic potential (metabolic diversity) and hydrocarbon specificity of hydrocarbon-degrading communities [48]. In the bacterial consortia, the antagonistic effect of some other bacteria could limit the efficiency of bacterial remediation [67]. Hence, the activities of these antagonistic bacteria have to be controlled to encourage the continued development of viable potential bacterial consortia. Many studies showed that microbial communities (bacteria, fungi, yeasts, and algae) play a great role in the biodegradation of hydrocarbon pollutants [56, 65]. There are several bacterial genera that are tested for the degradation of hydrocarbon (Table 2). The most known hydrocarbon degrader bacteria belong to the genus of *Pseudomonas*, *Bacillus*, *Sphingomonas*, *Rhodococcus*, *Alcaligenes*, and so on [7, 24, 27, 63, 68–71]. Those bacteria can usually metabolize only a limited range of hydrocarbon substrates. Some of them degrade alkanes (normal, branched, and cyclic paraffin) and aromatics while others both paraffinic and aromatic hydrocarbons [68]. Therefore, a bacterial consortium (mixing of potential bacteria) with overall broad enzymatic capacities is required to synergistically degrade complex mixtures of pollutants. Supporting this, studies also showed that consortia of *Staphylococcus* sp., *Stenotrophomonas* sp., and *Pseudomonas* sp. [66]; *Pseudomonas* sp., *Acinetobacter* sp., and *Rhodococcus* sp. [49]; *Marinobacter* sp. and *Roseovarius* sp. [71]; *Pseudomonas aeruginosa* and *Rhodococcus erythropolis* [57]; *Bacillus* sp., *Corynebacterium* sp., and *Pseudomonas* sp. [65]; *Pseudomonas* sp., *Gordonia* sp., *Aeromonas* sp., *Stenotrophomonas maltophilia, Xanthomonas* sp., *Alcaligenes xylosoxidans*, and *Rhodococcus* sp. [72]; and *Stenotrophomonas* sp., *Bacillus* sp., *Brevibacillus* sp., *Nocardiodes* sp., and *Pseudomonas* sp. [70] indicated proficient bioremediation activities. However, the functioning of bacterial community structures and diversity for the complete bioremediation/biodegradation is influenced by hydrocarbon components, nutrients limitation upon competition (TC, TN, TP, and TOM), metabolites produced, soil physicochemical properties, and the compatibility of the mixture during bioremediation [73]. Therefore, using the consortium of potential microbes with overall broad catabolic enzymes and genes, biodegradation may become faster, efficient, and complete.

Table 2: Bacterial genus reported for hydrocarbon degradation.

S. No.	Bacterial genus	References
1	*Pseudomonas*	
2	*Cycloclasticus*	
3	*Sphingomonas*	
4	*Rhodococcus*	
5	*Neptunomonas*	
6	*Microbulbifer*	
7	*Polaromonas*	
8	*Cellulomonas*	
9	*Achromobacter*	
10	*Gordonia*	
11	*Marinobacter*	
12	*Actinobacteria*	
13	*Deltaproteobacteria*	
14	*Dietzia*	
15	*Alcaligenes*	
16	*Moraxella*	
17	*Mycobacterium*	
18	*Arthrobacter*	
19	*Pasteurella*	
20	*Haloarcula*	
21	Marinobacter,	
22	*Flavobacterium*	[7, 24, 27, 63, 68–71]
23	*Roseovarius*	
24	*Corynebacterium*	
25	*Bacillus*	
26	*Micrococcus*	
27	*Nocardia*	
28	*Xanthomonas*	
29	*Phanerochaete*	
30	*Vibrio*	
31	*Anabaena*	
32	*Serratia*	
33	*Altererythrobacter*	
34	*Burkholderia*	
35	*Alcanivorax*	
36	*Ralstonia*	
37	*Alkanibacter*	
38	*Paenibacillus*	
39	*Aeromonas*	
40	*Enterobacter*	
41	*Promicromonospora*	
42	*Microcella*	
43	*Stenotrophomonas*	

6.4.1. Biofilm Formation. To survive, bacterial cells form collection in the form of flocks, mats, or biofilm Biofilm weight (up to 90%) is composed of extracellular matrix and microbial cells [74]. When microbial communities encounter a harsh situation they react with any change of environment (stimuli) by producing extracellular polymers that surround them and facilitate their attachment on the substratum [75]. This is important to change response with respect to their growth, catabolic gene exchange, gene transcription and/or horizontal gene transfer, and an adaptive mechanism for physicochemically harsh conditions and regulate redox state of their environment [32]. Biofilm can be, however, formed by single or groups of microbial species, and their potential in hydrocarbon biodegradation is tested using some species of bacterial species such as *A. borkumensis*, *Pseudomonas* sp., *Marinobacter hydrocarbonoclasticus*, and *Bacillus* sp. [76]. Microbial communities that form biofilm are the potential to increase bioaccessibility of the carbon sources from the hydrocarbon-contaminated sites by synergistic effects, adapting to a new environment and acquiring metabolic changes to survive in an environment with a limited range of nutrients [75]. The formation of biofilm enhances biodegradation of hydrocarbon polluted soil environment by increasing microbial adaptation to low bioavailability of hydrophobic compounds [60]. This is due to the high microbial biomass within the biofilm being efficient for biosorption (to reduce immobilization of contaminants) as compared to the dispersed

microbial cell growth in the contaminated sites. Additionally, biofilm formation is vital to provide optimal factors (pH, salt concentration, and redox potential) for effective growth microbial communities and their hydrocarbon biodegradation [32]. Likewise, aggregation of multicellular entities embedded in matrices is important to facilitate hydrocarbon biodegradation by their involvement in flocculation and binding of heavy metals [77]. In addition, the exopolymer serves as a microbial attachment onto surface substratum, regulation of energy transfer and waste production, environmental protection (change in osmolality, temperature, adsorbs metals, and different organic compounds), and mechanical stability. This is because it consists of numerous biological components (polysaccharides, proteins, nucleic acids, lipids, and humic substances), all of which regulate the hydrophobicity, biodegradability, and adsorption properties of the biofilm [74]. Hence, the presence or absence of biofilm formation will enhance or hinder the hydrocarbon biodegradation rates. The more biofilm formation, the more biodegradation rate will be happening.

6.5. Number of Hydrocarbon-Degrading Bacteria. The number (population density) of hydrocarbon-degrading bacteria is a key factor for the degradation of hydrocarbon pollutants in the soil [56]. The number and diversity of potent hydrocarbon-degrading bacteria are relatively fewer than the number of whole naturally available bacteria if the site is not previously contaminated with hydrocarbon contaminants [78]. However, several hydrocarbon-polluted milieus are successfully conquered by hydrocarbon-degrading bacteria than other bacteria which do not have hydrocarbon degradation potentials. This variation is important to indicate that the hydrocarbon-degrading bacteria are known to possess effective hydrocarbon degradative enzymes and versatile metabolic pathways. Studies showed that for successful hydrocarbon biodegradation, the number of soil bacteria is usually in the range of 10^4–10^7 CFU per gram of soil, while lower than 10^3 CFU per gram of soil showed less biodegradation potential [56]. The high population of hydrocarbon-degrading bacteria substantially leads to more degradation rate [64]. Therefore, for having effective bacterial remediation, it is important to determine the number of potential microbes in the contaminated sites.

6.6. Bacterial Metabolic Capability. Bacterial metabolic potential with diverse and appropriate metabolic pathways is a key factor for the degradation, transformation, and mineralization of various hydrocarbon pollutants in the soil [70]. Microbial species that can utilize toxic pollutants are becoming dominant in the contaminated sites [67]. They also develop efficient catabolic activities by producing intracellular or extracellular broad substrate-specific enzymes: oxidoreductases, oxygenases (monooxygenases and dioxygenases), dehydrogenases, hydrolases, peroxidases, peroxidases, and laccases [22, 33], and new metabolic pathways (genetic changes) to degrade hydrocarbon pollutants either aerobically or anaerobically [56]. The genes that encode hydrocarbon degradative enzymes are critical for the potential of microbes to bioremediate soils contaminated with hydrocarbon pollutants [60] and identified from various kinds of hydrocarbon-degrading Gram-positive and negative bacteria [16]. Such overall possibilities are controlled by catabolic plasmids (known as chromosomal or plasmid DNA) and codified by genes for specific physiological functions. The genes such as *assA, bssA, phe*, *nahAc*, *xylE*, *PAH-RHDα,* GN-RHDα, *phd*, *nag*, *phn*, *nar*, *nid*, *alk*B, *TOL, ndoB*, *alkM*, a*lkB1, alkB2, nahH*, *SAL, pND50,* Alma, C230, PAH-RHD(GP), nahAC, *pWW31, pJP1, pJP4, pKF1, pAC21, pRE1, pAC25, pWR1*, and *pCS1* are reported to encode many aromatic compound degradations [14, 55, 78, 79]. Those various genes were identified in various bacterial genera such as *Pseudomonas* sp., *Sphingomonas* sp., *Comamonas* sp., *Alcaligenes* sp., *Acinetobacter* sp., *Burkholderia* sp., *Rhodococcus* sp., *Nocardioides* sp., *Mycobacterium* sp., *Klebsiella pneumoniae*, *Enterobacter cloacae, Bacillus pumilus*, and *Burkholderia* sp. [18, 79].

Most hydrocarbon-degrading bacteria, on the other hand, are unable to catabolize all types of hydrocarbon compounds. This is due to the fact that they have specialized hydrocarbon-degrading enzymes. As a result, some can digest aliphatic hydrocarbons, while others can only break down aromatics (mono and/or poly), with just a few microbes possessing physiologically diverse metabolic capacity [59]. For metabolically active bacteria, aerobic and anaerobic hydrocarbon metabolisms are studied [80]. For aerobic metabolic pathways, hydrocarbon-degrading bacteria are known to use either monooxygenase (incorporate one O_2 atom) or dioxygenase (incorporate two atoms of O_2) enzymes for the metabolism of aliphatic and aromatic hydrocarbons. Most microorganisms use monooxygenases to oxidize alkanes by attacking terminally, biterminally, or subterminally for the production of alcohol and further oxidize to aldehyde and fatty acid and finally get into Kreb's cycles via β-oxidation process [80, 81]. Some other potential microorganisms use dioxygenases (alkane monooxygenase, ring-hydroxylating dioxygenases, and catechol dioxygenase) to degrade aromatic hydrocarbons using either *ortho* or *meta* ring fission mechanisms, and the products are further metabolized to tricarboxylic acid cycle intermediates and sooner or later mineralized to CO_2 and H_2O [18]. For anaerobic pathways, both aliphatic and aromatic hydrocarbon contaminants can be oxidized into phenolic compounds or organic acids and then changed into a long chain of volatile fatty acids to end with methane and carbon dioxide [80]. Consequently, microbial catabolic activities are the major factors limiting or promoting biodegradation depending on the type of metabolic pathways that the microbes enjoy and the nature of the contaminants. Additionally, the numbers of different bacterial genes are important to judge the contamination level and natural degradation capacity.

6.7. Redox Potential of the Bacteria. Bacteria require energy for biological functions, cell maintenance, and reproduction. Physiologically, this energy is produced within bacterial cells via a redox reaction [51]. Bacteria, therefore, catabolize

hydrocarbon pollutants as the source of energy by catalyzing the transfer of electrons from electron donors to electron acceptors in aerobic or anaerobic bioremediation. This phenomenon depends on redox potentials that are important to drive the oxidation of hydrocarbon-derived pollutants for bacterial energy production (respiration) and enhance their growth by reducing the redox potential. Studies showed that many organic pollutants like hydrocarbon have a low rate of degradation due to their high redox potential [82]. Thus, the redox potentials show oxidizing or reducing conditions, provide an indication of the relative dominance of the electron acceptor (oxygen, nitrate, sulfate, iron (III), manganese, chlorate, perchlorates, etc.) [8, 51, 83], and require an energy source (electron donor) and nutrients for bioremediation processes. For aerobic conditions, oxygen does not only act as a final electron acceptor but is also important to enzymatic activation of aromatic hydrocarbon as mono or dioxygenases. As a result, aromatic compounds are favorable electron donors for bacterial growth because of the high Gibbs free energy change of the oxidation of these compounds with different electron acceptors [83]. In addition, for anaerobic conditions, the substances that have high reduction potential (e.g., perchlorate and chlorate) make them ideal electron acceptors for microbial metabolism during bioremediation processes.

When hydrocarbon-degrading bacteria grow well with decreasing pollutant, content is associated with the redox potential of the electron acceptors [84]. In highly contaminated sites, the electron donors (hydrocarbon) are excess over the oxidation potential of electron acceptors. Consequently, the respiration and biodegradation process can be controlled by the availability of specific electron acceptors [85]. Accordingly, bacterial degradation can be limited due to the low redox potential and depletion of electron acceptors. This is because the lower redox potential results in anoxic conditions and reduces the rate of biodegradation [86]. In general, the presence of dominant electron acceptors in hydrocarbon-contaminated sites is an important factor affecting the rate of biodegradation.

6.8. Effect of Biosurfactant. When hydrocarbon pollutants are released into the soil, they become difficult to biodegrade. This is due to their firm adsorption to soil matrices and less or no bioavailability for microbial biodegradation [67]. To overcome this problem, the uses of inorganic and organic surface-active agents have been exercised [7, 87]. The chemically synthesized surfactants have been used to improve the solubility of hydrocarbons through the process called emulsification. For this, treatment hydrocarbon contaminants using ionic (anionic and cationic), nonionic, biological, and mixed surfactants were used [2]. However, chemically synthesized (inorganic) surfactants were not recommended for further use since they are also prone to secondary contamination and are mostly hazardous to the environment with minimal or no effect on hydrocarbon biodegradation efficiency [71]. This indicates that the use of biosurfactants obtained from natural sources (plants or microorganisms) showed efficient activities and is acceptable since they are biodegradable, nontoxic, specificity under extreme circumstances, and more appropriate for hydrocarbon degradations [50]. As part of this, hydrocarbon-degrading bacteria produce those surface-active biological molecules extracellularly to emulsify and easily uptake the hydrocarbon contaminants [32, 57, 81, 88]. There are many kinds of biosurfactants such as glycolipids (rhamnolipids, emulsans, liposans, sophorolipids, and trehalolipids), lipopeptides and lipoproteins, surfactin, lichenysin, fatty acids, polymeric biosurfactants, phospholipids, and neutral lipids. They are produced from various microbes such as *Bacillus* sp., *Pseudomonas* sp., *Aeromonas* sp., *Enterobacter* sp., *Burkholderia* sp., *Acinetobacter* sp., *Micrococcus* sp., *Rhodococus* sp., and some halophile species [33, 57, 69, 81, 88, 89].

Biosurfactant enhances hydrocarbon degradation in many ways, including by modulating the solubilization and desorption of pollutants as well as modification of bacteria cell surface properties. Biosurfactants are capable of lowering the surface tension and the interfacial tension of the water/air or water/oil interface [32]. Thus, the surface area of the hydrocarbon substrate increases, making emulsification easier, and the entire phenomena make the substrate to be readily available for uptake and metabolism [55]. Additionally, the reduction of the interfacial tension leads to increased penetration of porous materials via the aqueous phase. Biosurfactants forms micelles in aqueous solutions at a concentration exceeding critical micelles concentration (CMC) [19]. To display the lowest surface tension, the hydrophobic part boosts the exploitation and solubilization of hydrocarbon contaminants into the solution. However, the CMC of surfactants and their effectiveness for the removal of hydrocarbon pollutants may be entirely dependent on their nature, type, composition, ionic strength, the physicochemical characteristics of the reaction mixture, and other factors [2]. In addition, the uses of biosurfactants also enhance the expression of siderophores production (metal-binding peptides) and biofilm formation for hydrocarbon biodegradation [77]. Overall, biosurfactants are capable to increase the solubility, mobility, dispersion, bioavailability, and degradability of hydrocarbon pollutants [3]. Therefore, for efficient hydrocarbon bioremediation, fulfilling and optimization of the bacterial growth parameters are not enough without consideration of the capacity of the bacteria to produce appropriate biosurfactants.

7. Abiotic Factors

7.1. Hydrocarbon Characteristics

7.1.1. The Physical and Chemical Nature of the Contaminant. Microbial bioremediation strategy is always needed to infer the interaction between the potential microbes and the hydrocarbon pollutants. The physical (heaviness, occurrence, diffusion rate, and viscosity) and chemical properties (components, molecular weight, and structures) of the hydrocarbon pollutants can affect biodegradation, transportation, and metabolism of a single strain or consortia of bacteria. This is due to the molecular size, composition, structure, concentration, toxicity, and unpredicted products

of hydrocarbon in the contaminated environment. These conditions affect the occurrence, stability, and biological activities of hydrocarbon-degrading bacteria. In addition, the high molecular weight of polyaromatic hydrocarbons (four or more rings: pyrene, chrysenes, fluoranthene, benzo [a] pyrene, and coronenes), and highly condensed cycloalkane compounds are recalcitrant than unbranched alkanes (intermediate length: C_{10}–C_{25}) and lighter PAHs (two or three rings: naphthalene, phenanthrene, and anthracene) to microbial degradation [77, 90]. This means the rate of hydrocarbon biodegradation of *n*-alkanes > branched alkane's > low-molecular-weight aromatics > high molecular weight of aromatic hydrocarbons > asphaltenes [8, 80]. The nondegradation prospective of the contaminant is because their nature accounts for their solubility, bioavailability, toxicity to microbes by the disruption of the lipid membrane, and unable to cleave the ring for degradation. Therefore, understanding the chemical characteristics, physical state (heterogeneity and degree of spreading), and toxicity, and the fate (physical, chemical, and biological change) of hydrocarbon contaminants is mandatory to determine the rate of hydrocarbon biodegradation [56].

7.1.2. Hydrocarbon Concentration. The concentrations of hydrocarbon pollutants affect the rates of uptake, transformation, and mineralization of bacterial biodegradation. Extremely high concentrations are known to be toxic and negatively influence the growth rate and biomass production of degraders and require a long treatment duration [91] due to the heavy and undispersed nature of high concentration pollutants [66]. In addition, it was confirmed that hydrocarbon concentration >5% decreases microbial degradation activity and may interrupt C:N:P ratio and oxygen availability [54]. Similarly, extremely low concentrations of hydrocarbons limit biodegradation by suppressing bacterial metabolic genes not to produce degradation enzymes and causes low supply or unavailability of carbon to support microbial growth [51]. Therefore, bacterial biodegradation of hydrocarbon depends on the presence of optimum concentration (not too high or low) for complete mineralization from contaminated environments.

7.1.3. Bioavailability of the Hydrocarbon Contaminants. According to Maletić et al. [47], the term bioavailability is defined in many ways: it refers to an interactions between pollutants and living cells, the degree to which pollutants in the soil may be absorbed or metabolized, maximum quantity of a contaminant available for uptake by an organism within a given time period, and the chemically active compound on its way to a degrader organism. In general, the bioavailability of hydrocarbon pollutants refers to the number of hydrocarbon substrates that are abundantly accessible to potential microbes [4, 58], and it is a rate-limiting factor in biodegradation processes [3]. However, the bioavailability is of pollutants can be restricted because of their high hydrophobicity, low water solubility (high molecular weight), low concentration, low diffusion rate, desorption onto soil and inorganic matrix colloids, chemical structures, temperature, viscosity, duration of the contamination, and soil characteristics (particle size and types of soil) [7]. For instance, at low temperature, viscosity and solubility of pollutant decreases; ice formation in the soil increases; and the transfer of oxygen, nutrients, and hydrocarbon in the soil (bioavailability) decreases. These lead to low accessibility for bacterial metabolism. Hence, the lack of bioavailability of hydrocarbon contaminants in the soil is a major factor inhibiting the rate of their biodegradation rate. This rate-limiting factor can be partially overcome by the use of biosurfactants, which increase the bioavailability of hydrocarbons. In a few cases, however, the biosurfactants can inhibit bacteria, and thus, the effect of the biosurfactant depends on the physicochemical properties of the biosurfactants, the types of pollutants, and the physiological properties of the functional microorganisms [27]. In this view, selection of biosurfactants is important, and several species of bacteria, such as *Bacillus* sp. DQ02 and *Bacillus amyloliquefaciens* An6, showed improved degradation efficiency with biosurfactants [19].

7.2. Physical Parameters

7.2.1. Soil Characteristics. The soil is the most multifaceted environment to harbor various kinds and populations of microorganisms. Its environmental factors (physicochemical properties) are the determinant stimuli for the nature of indigenous microbes, bacterial community, and the composition of functional genes [73]. The soil physicochemical characteristics that show such effect include soil type, region, texture, particle size, maximum water holding capacity (moisture), temperature, nutrient content, oxygen content, and pH [6, 91]. Overall, those soil factors are affecting the chemical stability, bioavailability, and movement of hydrocarbons pollutants in the soil to support potential bacterial growth and then ultimately for effective biodegradation [11].

7.2.2. Soil Region. The topsoil (surface soil, vadose zone, and unsaturated zone of soil) predominantly harbors a high population of bacteria due to the regular supplement of organic matters (from plants and animals) and adequate oxygen availability. Contrarily, in sediments (saturated zone of soil), there is a low bacterial population due to the decrease in organic matter supplement and oxygen availability with an increase in soil depth [59]. Therefore, the soil region affects the number and community of bacterial population in the hydrocarbon-contaminated soil.

7.2.3. Soil Particle Size. The size of the soil particle also determines the soil permeability and rate of hydrocarbon biodegradation [92]. Fine soil particles (clay soil; with small interstitial spaces) retain the hydrocarbon at the soil surface and reduce the availability of nutrients and oxygen whilst coarse soil particles (sand soil; with large interstitial spaces) drain hydrocarbon pollutants through the soil to the unsaturated zone. Those properties of soil make the rate of

hydrocarbon biodegradation very sluggish [6]. Hence, for efficient and rapid biodegradation, abstemiously drained soil particles (increase in porosity) are important for intensification of the contaminant's bioavailability, supply of oxygen, and enhancing the metabolism and growth of potential indigenous microbes [22].

7.2.4. Nutrient Availability. Bacteria require nutrients for their metabolism and growth, and therefore, the microbial community is dependent on nutrient accessibility in the hydrocarbon-contaminated natural soil environment [5]. In a natural environment, the hydrocarbon-degrading bacteria are regulated by inorganic nutrients as limiting factors [62]. This implies that the presence or addition of macronutrients (N, P, and K) in the contaminated soil increases the biostimulation [91] and determines the distribution and degradation of hydrocarbon pollutants [65]. Organic matter in the soil is a source of nutrients and enhances some properties of soil to promote the growth and activities of soil microbes [93]. Thereby, treatment of the soil with poultry manure alone can enhance oil degradation [94]. Besides, a high accumulation of organic nutrients leads to the toxicity of soils due to the production of toxic intermediates during hydrocarbon biodegradation [95]. Even though those hydrocarbon-degrading bacteria use hydrocarbons as an excellent source of carbon and energy, access to other nutrients such as nitrogen and phosphorus is limited from the contaminant itself. When there is a large accumulation of organic carbon contents in the hydrocarbon-contaminated sites, there is the rapid depletion of other inorganic nutrients such as nitrogen, phosphate, and potassium (N, P, and K) and trace amounts of calcium, sulfur, magnesium, iron, and manganese due to rapid microbial metabolic activities. This limits the rate and extent of hydrocarbon biodegradation and biotransformation. To avoid such limitation, studies recommended adjustment of carbon/nitrogen/phosphorous (C/N/P) ratio to 100:10:1 [96] to 100:20:1 [58, 91] for considerable hydrocarbon biodegradation. This can be applied by the addition of urea, phosphate, N–P–K fertilizers, and ammonium, and phosphate salts are mandatory [56]. It has been demonstrated that concentrations of phenanthrene did not change significantly without supplement of inorganic fertilizer (nitrogen and phosphorous) but decreased greater than 25 times in soil amendment [79]. Even though some bacteria exhibit good growth or adaptation for the high amount of carbon, nitrogen, potassium, and phosphorus sources in hydrocarbon-contaminated sites, biostimulation (extra supplement of nutrients or fertilizers) significantly affects indigenous microbial communities [12]. This reduces the rate of degradation by suppressing microbial growth and production of enzymes and ultimately resulting in toxicity to soil microbes [29, 61]. In addition, high concentration hydrocarbon contaminants could also alter the NPK ratio and thus ultimately resulting in oxygen shortage. In general, improper supply (excessive or low) and/or absence of mineral nutrients limit the growth of hydrocarbon utilizing bacteria in the soil. Therefore, the supplement of optimal level of nutrients (N and P) in contaminated soils is indispensable for effective hydrocarbon biodegradation [25, 64, 92]. The hydrocarbon biodegradation is, therefore, depends on soil environment amendments, and in that way, the maximum degradation rate can be enhanced with the addition of nutrients to the optimal level.

7.2.5. Aeration/Oxygen Availability. Hydrocarbon degrading bacteria (oleophilic) can respire with the presence of oxygen (aerobic) and the absence of oxygen (aerobic or facultative). The availability of molecular oxygen in the soil is the limiting factor for aerobic bioremediation and acts as the final electron acceptor [28] and chemical reactant (the enzymatic oxygenase reactions) for a terminal, subterminal, and biterminal oxidation and ring cleavage of aromatic hydrocarbon contaminants [15]. The study indicated that without considering the total biomass of potential hydrocarbon-degrading bacteria, 3.1 mg/ml of oxygen is required for the degradation of 1 mg/ml hydrocarbon contaminants [54], and 10–40% of oxygen level is required for effective hydrocarbon biodegradation [28]. It is, therefore, aerobic catabolism results higher biodegradation rate than anaerobic metabolism [97, 98]. Reports showed that the use of isolated bacterial strains degraded 20–25% of the total amount of oil under aerobic conditions within 10 days, but the same strains took 50 days under anaerobic conditions to degrade 15–18% of the total petroleum present [68] and no or diminutive hydrocarbon pollutant degradation in anoxic soil region [77]. However, the low level of oxygen in hydrocarbon-contaminated sites hampers the aerobic degradation routes and resulting low removal efficacy [30]. The factors resulting in slow biodegradation and free oxygen depletion in the soil include oxygen concentration, bacterial oxygen consumption rate, physical and chemical properties of contaminated soils (type and porosity), and the presence of utilizable substrates or accumulation of organic matters [6, 28]. Therefore, the availability of oxygen in the soil is essential to have effective biodegradation.

7.2.6. Effect of Temperature. The temperature has a significant effect on the rate of bacterial growth, the activity of the enzymes, the chemistry of the pollutants, physiology, and diversity of the bacterial community responsible for degradation processes [15, 51]. Thus, temperature is considered an important factor in biological remediation, and the efficiency of removing hydrocarbon pollutants is directly related to temperature. Liu et al. [62] reported that temperature played a significant role in selecting petroleum hydrocarbon-degrading bacteria in deep and surface waters of the Northern Gulf of Mexico, besides water chemistry and the initial microbial community. The community of bacteria associated with hydrocarbon degradation is linked to temperature fluctuation and is that moderate temperatures are generally attributable for selecting mesophilic hydrocarbon-degrading bacteria. Several hydrocarbon-degrading bacteria were also shown to have a wide range of degradation effectiveness, suggesting the importance of temperature in remedial methods for the remediation of hydrocarbon contamination [27].

Additionally, studies indicated that the rate of hydrocarbon degradation is dependent on the temperatures of the region (tropical, temperate, and arctic) of contaminated sites [31]. For instance, psychrophiles require below 20°C; mesophiles require between 15–45°C; and thermophiles require above 50°C for hydrocarbon metabolism [56]. As temperature increases, solubility, bioavailability, diffusion, and enzymatic activities of microorganisms also increase [98], whereas, at a higher temperature (above 40°C), oxygen solubility decreases, and metabolic activity of aerobic bacteria diminishes; the membrane becomes more susceptible to hydrocarbon toxicity and consequently reduces the hydrocarbon biodegradation rate [56, 68]. Conversely, at lower temperatures, the oil viscosity increases; the volatility of low-molecular-weight of toxic hydrocarbons pollutants decreases; solubility decreases; the toxicity of low-molecular-weight of hydrocarbons increases; rates of enzymatic activity decreases; and hence, there is a delay microbial biodegradation [52]. Indeed, the degradation efficiency of hydrocarbons at lower temperatures could be improved with biosurfactants by influencing the viscosity and solubility of hydrocarbons. In addition, studies have shown that the maximum rates of microbial hydrocarbon degradation occur in a warm environment [31], particularly in the range of 30–40°C [52, 97]. In general, the rate of biodegradation decreases with decreasing temperature, while it accelerates with increasing temperature. As a result, potential bacteria need an optimal temperature range for complete hydrocarbon degradation.

7.2.7. Effect of pH. Soil pH (neutral, acidic, or alkaline) affects bacterial growth and bioremediation strategies. Most hydrocarbon-degrading bacteria favor neutral pH [98] to slightly alkaline [97]. The production and accumulation of bacterial waste products can change the pH of hydrocarbon-contaminated soils and consequently influences nutrient availability, solubility contaminant, bioavailability, and microbial activities. Studies showed that hydrocarbons can be minimally degraded with a range of pH 2–5.5 and alkaline pH 7.5–10 [54, 56], while effective hydrocarbon degradation is obtained at nearly neutral pH (5.2–7.0) [79] and the greatest bacterial population observed at pH 7.5 too [99]. Another study also recommended that for optimum hydrocarbon bioremediation soil, pH range will be 6–8 [98]. From the literature, it is found that a little change in pH can dramatically affect the rate of hydrocarbon biodegradation [61]. This is because the optimum soil pH is important to regulate microbial biomass and enzyme activity and boost hydrocarbon biodegradation [99]. Hence, it is important to adjust the soil pH in hydrocarbon-contaminated sites shall optimally ranging from 5 to 8 for having the greatest effect of bioremediation.

7.2.8. Effect of Moisture Availability. Soil moisture (water film) serves as the transport medium for soil nutrients and the removal of bacterial metabolic waste products in the soil particles. It affects hydrocarbon bioavailability, aeration status, nature and amount of soluble materials, osmotic pressure, diffusion processes, transfer of produced gases, soil toxicity level, and the pH of the soil [92, 93]. When the soil has hydrocarbon contaminants, its porosity and water holding capacity are reduced [22]. Ultimately, this condition decreases microbial activity since bacterial and soil water activities are directly proportional, that is, as moisture content decreases, bacterial activities too decrease, and contrariwise, when the soil moisture level is high, it limits oxygen transfer [63]. That is why hydrocarbon degradation in terrestrial ecosystems is limited by the availability of water for microbial growth and metabolism. Therefore, the proper moisture availability is essential for hydrocarbon biodegradation and in the range of 50–75% [95], 30–90% [98], and 50–80% [21]. However, extreme moisture conditions are unfavorable for microbial growth and metabolism. This is because the diffusion of oxygen in the soil is decreased and aerobic hydrocarbon degradations are limited rather than making anaerobic soil environments. Hence, providing proper moisture (water availability) in hydrocarbon-contaminated sites is mandatory to boost microbial activities for hydrocarbon degradations.

7.2.9. Effect of Salinity. The deposition and accumulation of salt naturally or anthropologically affect microbial hydrocarbonoclastic activity and increase stress for organisms (halophile or halotolerant) in their habitat [69]. High salt concentration can increase osmotic pressure and hinder microbial metabolic activities, solubility, and transportation systems for essential ions [55] and bring a lack of oxygen for microbes [97]. This might be happened due to the introduction of a large number of soluble fertilizers (nitrates or ammonium salts) and discharging of saline solution into soil. When such hypersaline environments are contaminated with petroleum compounds, halophilic bacteria have the potential to survive and degrade the pollutants. Those potential bacteria have genes encoding hydrocarbonoclastic enzymes, which are responsible for their versatile hydrocarbon catabolism [69]. Studies indicated that significant hydrocarbon degradation was detected with 0.1–2 M NaCl but maximally 0.4 M and a high level of soil salinity decreased the rate and extent of hydrocarbon biodegradation [56]. In addition, the supplement of salts such as NaCl and NH_4Cl in nutrient scarce soil enhances the microbial degradation of hydrocarbons [96]. This is because of the presence of such salts, hydrocarbon biodegradation in soil increased with increasing cation concentrations. However, the higher cation concentrations impede the microbial consortium in the soil and reduce microbial biodegradation of hydrocarbon contaminants [100]. Therefore, identification of proper salt concentration depends on hydrocarbon-contaminated soil environments and the nature of potential hydrocarbon-degrading bacteria.

8. Summary and Future Outlook

Hydrocarbon contaminants are considered a serious issue among environmental pollutants due to their high toxicity to human and environmental health. Several studies have shown that microorganisms can break down hydrocarbons utilizing a wide range of enzymes. The process can start in

contaminated sites whenever biotic and abiotic factors permit. As a result, it is essential to understand the role of biotic (the number, type, and composition of bacterial populations, microbial completion, bacterial redox potential, biosurfactant production, and genetic factors) and abiotic factors (the physicochemical properties of contaminant and environmental variables including bioavailability, nutrient, temperature, pH, aeration, and salinity) for effective contaminant removal. Therefore, for having effective hydrocarbon biodegradation, it is important to optimize environmental factors to allow optimum bacterial growth and metabolism. Furthermore, developing novel biosurfactants that improve the bioavailability of hydrocarbons to bacteria seems to be a promising strategy for overcoming the barrier to microbial absorption of petroleum hydrocarbons. Even though several rate-limiting factors have been elucidated in the current review, the interactive nature of microorganisms, hydrocarbons, and environment still is not completely understood, and further research is necessary to improve predictive understanding of the fate of hydrocarbon pollutants in the environment and the role of microorganisms in biodegradative environmental decontamination. Future research should also focus on establishing a microbial consortium capable of acting on a broad range of hydrocarbons, including saturates, aromatic (mono and polycyclic), resins, and asphaltenes. Various molecular tools such as metagenomics could help uncover the community assemblage residing in unusual hydrocarbon-contaminated environments. Ultimately, synthetic biology technology may be used to produce engineered bacteria with enhanced metabolic abilities and greater potential to break down hydrocarbons pollutants and thus ameliorating the current time-consuming hydrocarbon bioremediation process.

Data Availability

The data supporting this review are from previously reported studies and data sets, which have been cited.

Conflicts of Interest

The authors declare no conflicts of interest.

Acknowledgments

The authors wish to acknowledge all who had been instrumental in the creation of this review article. This review work did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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Hindawi
Journal of Chemistry
Volume 2021, Article ID 5286278, 11 pages
https://doi.org/10.1155/2021/5286278

Research Article

Lead and Chromium Immobilization Process Subjected to Different Freeze-Thaw Treatments in Soils of the Northeastern Qinghai-Tibet Plateau

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Received 3 August 2021; Revised 17 September 2021; Accepted 7 October 2021; Published 21 October 2021

Academic Editor: Peng Zhang

The freeze-thaw cycle is one of the important processes that affected heavy metal behaviors in soil. However, information regarding the adsorption and desorption behavior of heavy metals in soils under different freeze-thaw conditions is relatively less. Therefore, different freeze-thaw conditions including unfrozen, 15 freeze-thaw cycles at 60% water content, and 15 freeze-thaw cycles at 100% water content were investigated. Then the adsorption and desorption behaviors of Pb and Cr in freeze-thaw soils were studied. Results showed the Pb and Cr adsorption amount mostly decreased with increasing water-soil ratio, and the soil performance of Pb and Cr adsorption at same water-soil ratios showed variation under different freeze-thaw conditions. The Pb isothermal adsorption was higher for most freeze-thaw treatments compared to the control. The soil performance of Cr isothermal adsorption showed variation under different freeze-thaw conditions. Most electrostatic binding of Pb and Cr were stronger under unfrozen and freeze-thaw conditions than unfrozen conditions. Most Pb and Cr adsorption kinetics patterns of freeze-thaw treated soils were rapid than unfrozen conditions. These results implied that freeze-thaw cycles could change the soil adsorption and desorption patterns of Pb and Cr. Therefore, further studies are urgently needed to investigate Pb and Cr immobilization mechanisms in soils during freeze-thaw cycles. Hence, these findings provided useful information on Pb and Cr immobilization process in soils that underwent freeze-thaw cycles to offer an additional insight into predicting Pb and Cr behaviors in cold and freezing environments.

1. Introduction

The soil freeze-thaw cycle (FTC) is a common natural phenomenon in the high- and middle-latitude regions of the northern hemisphere; the Qinghai-Tibet Plateau is located in the temperate zone with an alpine climate [1]. Significant effects of freeze-thaw cycles on soil characteristics have been widely reported [2–4]. For example, freeze-thaw cycles (FTCs) can significantly alter soil aggregate stability and density [5, 6]. Soil community structures, microbial activities, and extractable N concentration are also affected by FTCs [7–10]. Organic mineralization can also be affected by FTCs [11]. Multiple freeze-thaw treatments (FT-treatments) also caused a significant increase in the release of dissolved organic carbon [12]. However, different experiment results have been found. The methodological differences in FTC experiments in different soils cause the different research results. Large FTC amplitudes and rapid FTC rates are reported to produce large effects on soil properties, whereas moderate FTC amplitudes and slow FTC rates have minimal effects [13, 14]. Additionally, the freeze-thaw time also differs significantly among different researches [15].

Compared with emerging contaminants, heavy metals might pose higher risks [16–18]. Heavy metal pollution

and emerging contaminants have attracted attention widely [19–22]. FTC is one of the critical processes that influence heavy metal behaviors through changing soil-specific surface area, organic matter content, physical structure, and other soil properties [23–25]. Many studies have demonstrated that the geochemistry behavior of some heavy metals can be altered by FTCs [26, 27]. It is generally regarded that adsorption and desorption of heavy metals in unfrozen soils were higher than the soil with FT-treatment [3, 27], but this study has different results. The soil adsorption amount of heavy metals increased with the decreased freezing temperature and FTC frequency [3, 28]. FTCs can also transport the particulate metals from soil environment to aquatic environment [25, 29]. Moreover, FTCs are important functions for the transform of heavy metals from organic or Fe-Mn oxides fractions to exchangeable fractions [30, 31]. However, most studies adopted extreme freeze-thaw conditions; for example, the researchers usually adopted −25°C as freezing temperature and 20°C as thawing temperature [25, 32]. The minimum temperature in a natural environment below −20°C rarely exists in soil when the FTCs occur. Therefore, the researchers should adopt a suitable temperature for the freeze-thaw conditions.

FTC is a typical climate characteristic in northwest China. Little information has been available on the adsorption and desorption of heavy metals in soils of Qinghai-Tibet Plateau experiencing FTCs. The immobilization processes of heavy metals in soil would be impacted by the property change caused by FTCs, which can cause variations in the soil environment. In this study, we took the soils of the northeastern Qinghai-Tibet Plateau in China as the research objects and probed into the behaviors of the adsorption and desorption of Cr and Pb in soils treated with different freeze-thaw conditions. The main targets of this were to (1) investigate isothermal adsorption and desorption behavior of Pb and Cr in the freeze-thaw treated (FT-treated) soils and (2) study kinetics of adsorption and desorption behavior of Pb and Cr in the FT-treated soils.

2. Materials and Methods

2.1. Site Description and Soil Samples. The experimental soils were sampled from top 20 cm layers in northeastern Qinghai-Tibet Plateau, China. The soil sampling sites included S-1 in background area (35.08°N; 97.75°E), S-2 in agricultural and pastoral area (36.98°N; 100.85°E), S-3 in industrial area (36.38°N; 94.94°E), S-4 in mining area (37.32°N; 95.55°E), S-5 in salt-lake area (36.75°N; 99.07°E), and S-6 in urban area (36.63°N; 101.75°E). Roots, stones, and vegetation were removed, and the soils were air-dried, mixed, and stored at 4°C in the dark until use.

The ground of the Qinghai-Tibet plateau is mainly classified into two major categories on the map of permafrost on the Qinghai-Tibetan Plateau (QTP 1996): permafrost and seasonally frozen [1]. The freezing-thawing phenomenon is distributed in this region widely. The freezing and thawing temperatures of the lab-simulated experiments were set as −15°C and +5°C, respectively.

2.2. Freeze-Thaw Procedure. About 200 g of soil samples were put into plastic bags. Soil moistures were adjusted to 60% and 100% water holding capacity. The freezing temperature (−15°C) and thawing temperature (+5°C) were selected to simulate the common winter temperature in northwest China. In the simulated experiments, the soil samples with different water contents were frozen under −15°C for 24 h and then thawed under 5°C for the next 24 h; the above processes were set as one FTC. The freeze-thaw processes of samples were conducted by a refrigerator (BCD-649WE, Qingdao Haier Co. Ltd., Qingdao, China). After 15 FTCs, samples of FT-treatment were air-dried and labeled as F15 + W60% and F15 + W100%, respectively. The unfrozen soil was kept under 5°C and labeled as F0 + W0%.

2.3. Pb and Cr Adsorption and Desorption Experiments. For each FT-treatment, about 1.0 g of treated soil sample was put into a 50 mL centrifuge tube. All the experiments were conducted at a fixed ionic strength ($NaNO_3$, analysis pure, Sinopharm Chemical Reagent Co. Ltd.). In order to illustrate the effect of water-soil ratio on adsorption of FT-treated soils, the solutions of 0.01 mol/L $NaNO_3$ containing 20 mg/L Pb^{2+} ($Pb(NO_3)_2$, analysis pure, Sinopharm Chemical Reagent Co. Ltd.) and 10 mg/L Cr^{6+} ($K_2Cr_2O_7$, analysis pure, DaMao Chemical Reagent Factory) were added to each tube. The Pb^{2+} and Cr^{6+} solution of volumes consisted of 10, 30, 50, and 80 mL, respectively. The Pb^{2+} contents of solutions were 0.2, 0.6, 1.0, and 1.6 mg, while the Cr^{6+} contents of solutions were 0.1, 0.3, 0.5, and 0.8 mg, respectively. Then the tubes were shaken at 180 times/min continuously for 2 h; the samples were centrifuged at 4000 rpm for 10 min and filtered through a 0.45 μm cellulose acetate syringe filter. The Pb and Cr concentration of the supernatants was measured by plasma emission spectrometer (Thermo ICAP 6500 DUO, Thermo Fisher, USA). The adsorption capacity of Pb and Cr were calculated as follows:

$$S_a = \frac{(C_0 - C_1)}{m} \times V, \quad (1)$$

where S_a is the adsorption capacity (mg/kg), C_0 is the initial Pb or Cr concentration in mg/L, C_1 is the Pb or Cr concentration of equilibrium (mg/L), V is the volume of added solution (L), and m is the weight of soil sample (kg).

For each sample, about 1.0 g of air-dried soil sample was placed in a 50 mL centrifuge tube. A 20 mL solution of 0.01 mol/L $NaNO_3$ containing varying concentrations of Pb and Cr was added to each tube. Solutions containing various concentrations (5, 10, 20, 50, and 100 mg/L) of 20 mL Pb^{2+} ($Pb(NO_3)_2$ solution) and various concentrations (5, 10, 20, 30, and 50 mg/L) of 20 mL Cr^{6+} ($K_2Cr_2O_7$ solution) were prepared. These experiments aimed to assess isothermal adsorption of FT-treated soils. Then the tubes were shaken at 180 times/min continuously for 2 h and then centrifuged at 4000 rpm for 10 min. The supernatants were filtered through a 0.45 μm cellulose acetate syringe filter.

Following the adsorption experiments of Pb or Cr, the centrifuged residues were weighed to calculate the content of residual solutions. Then 20 mL of 0.01 mol/L $NaNO_3$ solutions were added to the centrifuged residues. The tubes were shaken

for 2 h. The concentrations of Pb or Cr in the supernatant were measured as described above. The amount of desorbed Pb or Cr was calculated according to the following equation:

$$S_d = \frac{C_2 \times V - C_1 \times V_1}{m}, \quad (2)$$

where S_d is the desorption capacity in mg/kg, C_2 is the Pb or Cr concentration of equilibrium (mg/L), and V_1 is the volume remaining in the residue.

The isothermal adsorption behavior of Pb and Cr in the soil is calculated by the Freundlich and Langmuir models. The Freundlich model explains site adsorption intensity and heterogeneity between adsorbate and adsorbent [33]. The Freundlich equation has the following form:

$$\log Q_e = \log k + \frac{1}{n} \log C_e, \quad (3)$$

where Q_e is Pb or Cr adsorbed by soil (mg/kg), C_e is Pb or Cr concentration in adsorption equilibrium (mg/L), n is constant, and k is affinity coefficient (L/mg).

Langmuir model assumes that maximum adsorption corresponds to a saturated monolayer of solute molecules on the surface of the adsorbent [33], and the energy of adsorbance is constant. The Langmuir model is calculated according to the following equation:

$$\frac{C_e}{Q_e} = \frac{1}{Q_m} \times C_e + \frac{1}{k \times Q_m}, \quad (4)$$

where Q_e is Pb or Cr adsorbed by soil (mg/kg), C_e is Pb or Cr concentration in adsorption equilibrium (mg/L), Q_m is the adsorption maximum of Pb or Cr adsorbed by soil, and k is affinity coefficient (L/mg).

In order to illustrate the soil kinetics of adsorption, about 1.0 g of treated soil samples were put into a 50 mL solution centrifuge tube. The solutions of 0.01 mol/L $NaNO_3$ containing 20 mg/L Pb^{2+} ($Pb(NO_3)_2$ solution) and 10 mg/L Cr^{6+} ($K_2Cr_2O_7$ solution) were added to each tube. Supernatants were collected from the tubes at 5, 10, 30, 60, 240, and 480 minutes. The following experiment processes were the same as described above, and the concentrations of Pb^{2+} or Cr^{6+} in the supernatant were measured as described above.

The pseudo-first-order model is a conventional model examined to analyse the kinetics of adsorption processes. The pseudo-first-order kinetics model supposes that the diffusion step controls the adsorption and its adsorption is proportional to the difference in adsorption capacity at equilibrium and at any time [34]. The Weber–Morris model described intraparticle diffusion in the solid phase [34]. The pseudo-first-order (5) and Weber–Morris (6) models are described as follows:

$$\log(Q_e - Q_t) = \log Q_e - k_1 t, \quad (5)$$

$$Q_t = k_2 t^{1/2} + C, \quad (6)$$

where t is reaction time (h), Q_e and Q_t are adsorption capacity at equilibrium and at any time t (mg/kg), and k_1 and k_2 denote pseudo-first-order rate constant and intraparticle diffusion equation constant, respectively. C is a constant.

3. Results and Discussion

3.1. Effect of Water-Soil Ratio on Adsorption of FT-Treated Soils. The soil type in the sampling sites was sandy clay loam, and soil pH was an alkaline. The Pb and Cr concentrations of the selected soils would not affect the adsorption and desorption experiment because exchangeable Pb and Cr contents were under very low level. The soil performance of Pb and Cr adsorption at various water-soil ratios under different FT-treated are shown in Figure 1. The different soil adsorption trends of Pb and Cr responded more or less constantly with change in the water-soil ratios. The Pb and Cr adsorption amount mostly decreased with increasing the water-soil ratio. However, the soil performance of Pb and Cr adsorption at same water-soil ratios showed variation under different freeze-thaw conditions.

The FTCs with different freezing conditions exerted different effects on soils of the northeastern Qinghai-Tibet Plateau. At water-soil ratios of 10:1, 30:1, and 80:1, the Pb adsorption amount of soils in S-1 under different FT-treated (F15 + W60% and F15 + W100%) decreased compared to the unfrozen soil. At water-soil ratio of 50:1, the Pb adsorption amount in S-1 increased at 15 FTCs and 60% water content and decreased at 15 FTCs and 100% water content compared to the unfrozen soil. At water-soil ratio of 10:1, the Pb adsorption amount of FT-treated soils at 15 FTCs and 100% water content in S-2 was maximum value compared to the unfrozen and FT-treated soils (F15 + W60%), and the Pb adsorption amount of FT-treated soils at 15 FTCs and 60% water content in S-2 was maximum value (125.07 mg/kg and 9.9 mg/kg) at water-soil ratios of 30:1 and 80:1. The Pb adsorption amount of soils in S-2 under different FT-treated (F15 + W60% and F15 + W100%) decreased compared to the unfrozen soil at a water-soil ratio of 50:1. At a water-soil ratio of 10:1, the Pb adsorption amount of unfrozen soil in S-6 was maximum value (173.9 mg/kg), the Pb adsorption amount of soils under different FT-treated (F15 + W60% and F15 + W100%) decreased compared to the frozen soil. In the soils of S-3, the Pb adsorption amount of soils under different FT-treatments (F15 + W60% and F15 + W100%) decreased compared to the unfrozen soil at all the water-soil ratios; conversely, the soils in S-5 increased under different FT-treatments (F15 + W60% and F15 + W100%) at all the water-soil ratios. At different water-soil ratios, the Pb adsorption amount of FT-treated soils at 15 FTCs and 60% water content in S-4 was the maximum value compared to the unfrozen and FT-treated soils (F15 + W100%). At a water-soil ratio of 50:1 of soil in S-6, the changing trend was the same as a water-soil ratio of 10:1. At water-soil ratios of 30:1 and 80:1, the Pb adsorption amount in S-6 approached maximum values (159.36 mg/kg and 12.5 mg/kg) at 15 FTCs and 60% water content compared to the unfrozen soil; conversely, the Pb adsorption amount of FT-treated soil (F15 + W100%) in S-6 decreased compared to the unfrozen soil. Base on the experimental results, the soil performance of Pb adsorption at the same water-soil ratios showed variation under different freeze-thaw conditions.

The Cr adsorption patterns at the same water-soil ratios were different under different FT-treatments. At water-soil ratios of 10:1 and 50:1 of soil in S-1, the Cr adsorption

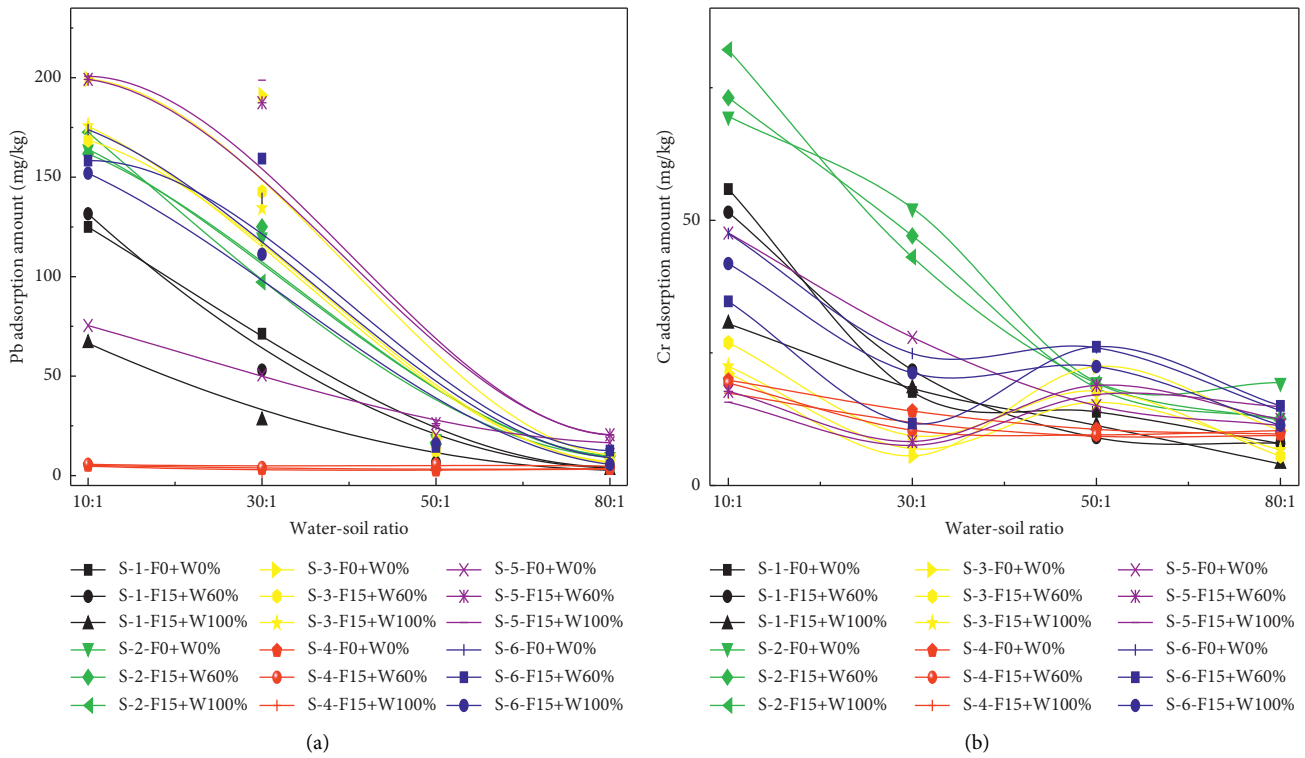

Figure 1: Relationship between water-soil ratio and Pb or Cr adsorption amount in soils under different freeze-thaw conditions. F0 and F15 refer to the number of freeze-thaw cycles (0 and 15 cycles), and W0%, W60%, and W100% refer to water ratio of field capacity (0%, 60%, and 100% water ratio).

amount of FT-treated soil in S-1 decreased compared to the unfrozen soil; conversely, the Cr adsorption amount of FT-treated soil increased at a water-soil ratio of 30:1. At a water-soil ratio of 80:1 in S-1, the Cr adsorption amount slightly increased at 15 FTCs and 60% water content and decreased at 15 FTCs and 100% water content compared to the unfrozen soil. At a water-soil ratio of 10:1 in S-2, the Cr adsorption amount of FT-treated soil increased compared to the unfrozen soil and decreased at water-soil ratios of 30:1, 50:1, and 80:1. At water-soil ratios of 10:1 and 30:1 in S-3, the Cr adsorption amount of FT-treated soil increased compared to the unfrozen soil and decreased at water-soil ratios of 50:1 and 80:1. At water-soil ratios of 10:1, 30:1, and 50:1 of soil in S-4, the Cr adsorption amount of FT-treated soil increased compared to the unfrozen soil. At water-soil ratio of 80:1 in S-4, the Cr adsorption amount slightly increased at 15 FTCs and 60% water content and slightly decreased at 15 FTCs and 100% water content compared to the unfrozen soil. At water-soil ratios of 10:1 and 30:1, the Cr adsorption amount of FT-treated soil decreased in S-5 compared to the unfrozen soil and increased at water-soil ratios of 50:1 and 80:1. At water-soil ratios of 10:1 and 30:1 of soil in S-6, the Cr adsorption amount of FT-treated soils in S-6 decreased compared to the unfrozen soil. At water-soil ratios of 50:1 and 80:1 in S-6, the Cr adsorption amount slightly increased at 15 FTCs and 60% water content and decreased at 15 FTCs and 100% water content compared to the unfrozen soil. The results indicated that Cr adsorption amount at same water-soil ratios was different under different FT-treatments.

3.2. Isothermal Adsorption and Desorption of FT-Treated Soils. Pb needs strict control since it can enrich in different matrices [17]. The adsorption and desorption of Pb in soils with different FT-treatments were shown in Figures 2(a), 2(b), 2(e), and 2(f)). A greater increase in adsorption was seen with increasing Pb concentrations from 0 to 20 mg/kg for the FT-treatments in the soils of S-1 and S-2, compared to adsorption with Pb concentrations ranging from 20 to 100 mg/kg. A greater increase in adsorption was seen with increasing Pb concentrations from 0 to 100 mg/kg for the most FT-treatments in the soils of S-3, S-5, and S-6. A greater increase in adsorption was seen with increasing Pb concentrations from 0 to 100 mg/kg for the treatment of 15 FTCs and 60% water content in S-4. A slight increase in adsorption was seen with increasing Pb concentrations from 0 to 50 mg/kg, and a greater increase ranging from 50 to 100 mg/kg for the treatment of 15 FTCs and 60% water content in S-4. The soil adsorption amount increased by the Pb concentration ranging from 0 to 50 mg/kg and decreased by the Pb concentration ranging from 50 to 100 mg/kg for the treatment of 15 FTCs and 100% water content in S-4. A high slope at Pb concentration illustrated a high proportion of available adsorption sites distribute on the surface of soil particles. In general, the adsorption of Pb was higher for most FT-treatments compared to the control. The desorption levels of Pb in soils by 0.01 mol/L $NaNO_3$ with different FT-treatments were shown in Figures 2(e) and 2(f)). About 0.08–10.52% (average value: 3.18%) of adsorbed Pb was desorbed from the soil without FTCs, while 0.02–10.67%

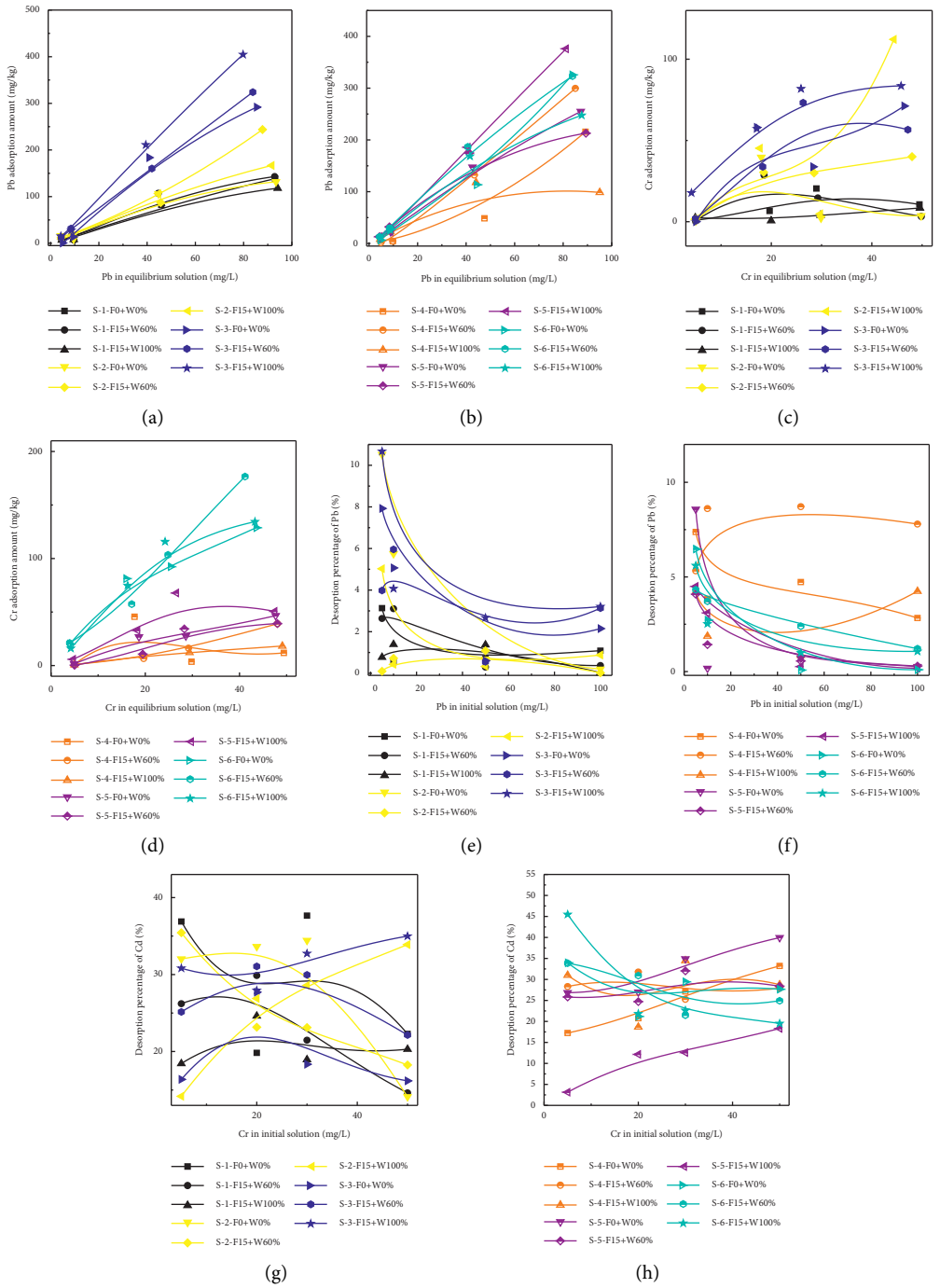

FIGURE 2: Adsorption amounts and desorption rates for Pb and Cr by soils under different freeze-thaw conditions. F0 and F15 refer to the number of freeze-thaw cycles (0 and 15 cycles), and W0%, W60%, and W100% refer to water ratio of field capacity (0%, 60%, and 100% water ratio).

(average value: 2.91%) of adsorbed Pb was desorbed from FT-treated soils at the same initial concentration of Pb. These data indicated that electrostatic binding of Pb in most soil samples was stronger under FTCs.

The Pb Langmuir and Freundlich parameters and data values are shown in Table 1. The $1/n$ in the Freundlich model is a measure of the heterogeneity of adsorption sites on the adsorbent surface. Surface site heterogeneity increases with $1/n$ approaches 0. Conversely, surface site homogeneity increases with $1/n$ approaches unity [35]. For the control with unfrozen soil, the $1/n$ value was higher at 0.87 ± 0.01 in soils of S-1, while the $1/n$ values for the FT-treatments (F15 + W60% and F15 + W100%) were lower at 0.78 ± 0.24 and 0.78 ± 0.21, respectively. This indicated that surface site homogeneity decreases with FT-treatment. The soils in S-4 and S-6 were similar to the soils of S-1 under the same FT-

Table 1: Freundlich and Langmuir parameters of Pb in soils under different freeze-thaw conditions.

Site area	FTCs	Pb (Freundlich)			Pb (Langmuir)		
		$1/n$	k	r^2	Q_m (mg/kg)	k	r^2
S-1	F0 + W0%	0.87 ± 0.10	2.69 ± 1.20	0.99	530.65 ± 244.75	0.003 ± 0.002	0.99
	F15 + W60%	0.78 ± 0.24	4.40 ± 4.72	0.93	319.98 ± 201.15	0.009 ± 0.009	0.95
	F15 + W100%	0.78 ± 0.21	3.51 ± 3.25	0.95	269.37 ± 144.31	0.008 ± 0.007	0.97
S-2	F0 + W0%	0.69 ± 0.23	6.08 ± 6.08	0.91	244.18 ± 116.17	0.01 ± 0.01	0.95
	F15 + W60%	1.47 ± 0.18	0.32 ± 0.26	0.99	—	—	—
	F15 + W100%	0.83 ± 0.13	3.96 ± 2.38	0.98	484.57 ± 211.89	0.005 ± 0.003	0.99
S-3	F0 + W0%	0.92 ± 0.22	4.92 ± 4.87	0.96	1181.38 ± 1453.03	0.003 ± 0.006	0.97
	F15 + W60%	1.02 ± 0.01	3.41 ± 0.28	0.99	—	—	—
	F15 + W100%	1.02 ± 0.08	4.68 ± 1.67	0.99	20390.80 ± 142821.15	$2.54E-4 \pm 0.001$	0.99
S-4	F0 + W0%	2.35 ± 0.13	0.005 ± 0.003	0.99	—	—	—
	F15 + W60%	1.31 ± 0.12	0.88 ± 0.498	0.99	—	—	—
	F15 + W100%	0.50 ± 0.30	11.69 ± 14.77	0.74	—	—	—
S-5	F0 + W0%	0.90 ± 0.10	4.55 ± 2.0	0.99	1158.05 ± 632.67	0.003 ± 0.002	0.99
	F15 + W60%	0.64 ± 0.20	12.37 ± 10.54	0.92	353.76 ± 105.46	0.01 ± 0.01	0.96
	F15 + W100%	1.08 ± 0.06	3.14 ± 0.94	0.99	—	—	—
S-6	F0 + W0%	1.57 ± 0.18	0.29 ± 0.24	0.99	—	—	—
	F15 + W60%	0.89 ± 0.10	6.12 ± 2.67	0.99	1413.49 ± 734.43	0.003 ± 0.002	0.99
	F15 + W100%	0.78 ± 0.16	7.81 ± 5.504	0.96	309.57 ± 1.53	$0.002 \pm 9.06E-5$	1.00

Note. "—" means that these data could not be fitted using the Freundlich and Langmuir models. F0 and F15 refer to the number of freeze-thaw cycles (0 and 15 cycles), and W0%, W60%, and W100% refer to water ratio of field capacity (0%, 60%, and 100% water ratio).

treatments; conversely, the $1/n$ values in soils of S-2 and S-3 under FT-treatments increased compared to the control with no FT-treatment. The results indicated that surface site homogeneity increased with FT-treatments in soils of S-2 and S-3. Compared to the control with no FT-treatment, the $1/n$ value was higher at 0.87 ± 0.01 under 15 FTCs and 100% water content in soil of S-5, while the $1/n$ values for the FT-treatment (F15 + W60%) were lower at 0.64 ± 0.20. The results indicate that different adsorption patterns were observed in different FT-treatments and different sampling sites.

The Q_m value in the Langmuir model represented the maximum adsorption capacity of Pb to the soil. According to Table 1, parts of data could not be fitted using the Langmuir model. Only the soils in S-1 under unfrozen and different freeze-thaw conditions could be all fitted the Langmuir model. The Q_m values indicated that maximum adsorption of Pb to the soil of S-1 decreased with FT-treatments. Maximum adsorption for the control was 530.65 ± 244.75 mg/kg, while Q_m values were 319.98 ± 201.15 mg/kg for FT-treatment (F15 + W60%) and 269.37 ± 144.31 mg/kg for FT-treatment (F15 + W100%). The K term in the Langmuir model equation indicates adsorption energy. As K values increase, the binding energy between soil colloids and ions increases. The results showed that K values of soils in S-1 increased under FT-treatments. The results suggested that the binding energy between soil colloid and Pb increased under FT-treatments.

The adsorption and desorption of Cr in soils with different FT-treatments are shown in Figures 2(c), 2(d), 2(g), and 2(h)). A slight change in adsorption was shown with increasing Cr concentration from 0 to 50 mg/kg for the most FT-treatments in the soils of S-1, S-4, and S-5. A greater increase in adsorption was seen with increasing Cr concentrations from 0 to 20 mg/kg for the most FT-treatments in the soils of S-2 and S-3, compared to adsorption with Cr concentrations ranging from 20 to 50 mg/kg. A greater increase in adsorption was seen with increasing Cr concentrations from 0 to 50 mg/kg for the most FT-treatments in the soils of S-6. In general, the adsorption of Cr had no regular pattern among these soil samples under different FT-treatments and unfrozen conditions. Desorption amount of Cr in soils by 0.01 mol/L $NaNO_3$ with different FT-treatments are shown in Figures 2(g) and 2(h)). About 14.11–39.95% (average value: 27.03%) of adsorbed Pb was desorbed from the soil without FT-treatment, while 3.13–45.47% (average value: 25.34%) of adsorbed Cr was desorbed from FT-treated soils at the same initial concentration of Cr. These results indicated that the electrostatic binding of Cr in most soil samples was stronger under freeze-thaw conditions.

The Cr Langmuir and Freundlich parameters in different soils under different freeze-thaw conditions are shown in Table 2. Compared to the control with no FT-treatment, the $1/n$ value was higher at 1.75 ± 0.81 under 15 FTCs and 100% water content in soil of S-1, while the $1/n$ values for the FT-treatment (F15 + W60%) were lower at 0.13 ± 0.82. For the control with unfrozen soil, the $1/n$ value was higher at 0.71 ± 0.51 in soils of S-3, while the $1/n$ values for the FT-treatments (F15 + W60% and F15 + W100%) were lower at 0.62 ± 0.47 and 0.50 ± 0.17, respectively. These results indicated that surface site homogeneity decreased with FT-treatment. Conversely, the $1/n$ values in soils of S-2, S-4, and S-6 under FT-treatments increased compared to the control with no

Table 2: Freundlich and Langmuir parameters of Cr in soils under different freeze-thaw conditions.

Site area	FTCs	Cr (Freundlich)			Cr (Langmuir)		
		$1/n$	k	r^2	Q_m (mg/kg)	k	r^2
S-1	F0 + W0%	0.54 ± 0.68	1.80 ± 4.39	0.40	—	—	—
	F15 + W60%	0.13 ± 0.82	7.97 ± 21.32	0.02	—	—	—
	F15 + W100%	1.75 ± 0.81	0.01 ± 0.02	0.80	—	—	—
S-2	F0 + W0%	0.34 ± 0.53	9.95 ± 18.12	0.27	—	—	—
	F15 + W60%	0.65 ± 0.28	3.30 ± 3.37	0.84	70.98 ± 40.99	0.02 ± 0.03	0.89
	F15 + W100%	—	—	—	—	—	—
S-3	F0 + W0%	0.71 ± 0.51	4.53 ± 8.33	0.65	—	—	—
	F15 + W60%	0.62 ± 0.47	6.18 ± 10.43	0.63	—	—	—
	F15 + W100%	0.50 ± 0.17	13.17 ± 7.78	0.88	124.76 ± 27.89	0.05 ± 0.02	0.95
S-4	F0 + W0%	0.11 ± 1.00	10.92 ± 35.26	0.01	—	—	—
	F15 + W60%	1.85 ± 0.08	0.02 ± 0.01	0.99	—	—	—
	F15 + W100%	0.883 ± 0.14	0.59 ± 0.32	0.97	76.20 ± 59.68	0.01 ± 0.01	0.98
S-5	F0 + W0%	0.86 ± 0.23	1.67 ± 1.45	0.93	172.81 ± 218.09	0.01 ± 0.01	0.93
	F15 + W60%	1.02 ± 0.46	0.78 ± 1.36	0.85	482.39 ± 3870.92	0.001 ± 0.01	0.85
	F15 + W100%	0.54 ± 0.42	7.41 ± 10.98	0.62	—	—	—
S-6	F0 + W0%	0.62 ± 0.11	12.62 ± 4.99	0.96	219.48 ± 43.91	0.03 ± 0.01	0.98
	F15 + W60%	1.13 ± 0.12	2.60 ± 1.152	0.98	—	—	—
	F15 + W100%	0.66 ± 0.18	11.73 ± 7.52	0.92	260.33 ± 93.79	0.02 ± 0.01	0.95

Note. "—" means that these data could not be fitted using the Freundlich and Langmuir models. F0 and F15 refer to the number of freeze-thaw cycles (0 and 15 cycles), and W0%, W60%, and W100% refer to water ratio of field capacity (0%, 60%, and 100% water ratio).

FT-treatment. These results indicated that surface site homogeneity increased with FT-treatments in soils of S-2, S-4, and S-6. Compared to the control with no FT-treatment, the $1/n$ value was higher at 1.02 ± 0.46 under 15 FTCs and 60% water content in soil of S-5, while the $1/n$ value for the FT-treatment (F15 + W100%) was lower at 0.54 ± 0.42. According to Table 2, most data of Cr adsorption could not be fitted using the Langmuir model. The results indicated that different adsorption patterns were observed in different FT-treatments and different sampling areas.

3.3. Adsorption Kinetics of FT-Treated Soils. In order to optimize the time required for access to equilibrium condition, a series of adsorption experiments were performed. This investigation also allowed verifying the kinetics of the process. The adsorption of Pb and Cr from aqueous solution to about 1 g of soil was studied by contacting the solid and liquid phases in the range of 5–480 minutes (Figure 3).

Base on pseudo-first-order model analysis, the equilibrium of adsorption of Pb ions was not achieved after 480 minutes for soil under unfrozen in S-1, 458 minutes for soil under 15 FTCs and 60% water content, and 376 minutes for soil under 15 FTCs and 100% water content. Equilibrium of adsorption of Pb ions was achieved after 3.33 h for soil under unfrozen in S-3, 0.72 h for soil under 15 FTCs and 60% water content, and 0.90 h for soil under 15 FTCs and 100% water content. Equilibrium of adsorption of Pb ions was not achieved after 480 min for soil under unfrozen in S-5, 0.70 h for soil under 15 FTCs and 60% water content, and 0.74 h for soil under 15 FTCs and 100% water content. These results demonstrated that the kinetics of Pb adsorption under FT-treatments were more rapid than the unfrozen soil in the S-1, S-3, and S-5. Equilibrium of adsorption of Pb ions was achieved after 7.84 h for soil under unfrozen and FT-treatment (F15 + W100%) in S-2; it was seen that the adsorption of Pb ions onto soil under 15 FTCs and 60% water content was more rapid than unfrozen and other FT-treatment. The adsorption of Pb ions onto soil in S-6 under 15 FTCs and 60% water content was more rapid than unfrozen and other FT-treatment. The results indicated that most Pb adsorption patterns on soils under FT-conditions were rapid than unfrozen conditions.

Table 3 lists the kinetic parameters for the models on the adsorption of Pb onto soils in six sites. R^2 for the Pb pseudo-first-order model ranged from 0.46 to 0.94. Most of the R^2 data were larger than 0.6, except soil in S-2 under unfrozen condition, soil in S-3 under FT-treatment (F15 + W100%), and soil in S-6 under unfrozen condition. R^2 for the Pb Weber–Morris model ranged from 0.02 to 0.92. Soils in S-4 and S-3 did not fit well in some conditions. Compared to the control with no FT-treatment, the Q_e value was higher at 25.41 ± 2.76 under 15 FTCs and 100% water content in soil of S-1, while the Q_e value for the FT-treatment (F15 + W60%) were lower at 20.46 ± 2.96. For the control with unfrozen soil, the Q_e value was higher at 41.05 ± 7.4 in soils of S-2, while the Q_e values for the FT-treatments (F15 + W60% and F15 + W100%) were lower at 28.35 ± 5.17 and 35.53 ± 4.69, respectively. The soils in S-5 were similar to the soils of S-2 under the same FT-treatments; conversely, the Q_e values in soils of S-3, S-4, and S-6 under FT-treatments increased compared to the control with no FT-treatment.

Equilibrium of adsorption of Cr ions was not achieved after 480 min for soil under unfrozen in S-5, 0.78 h for soil under 15 FTCs and 60% water content, and 0.51 h for soil under 15 FTCs and 100% water content. Equilibrium of adsorption of Cr ions was achieved after 0.74 h for soil under

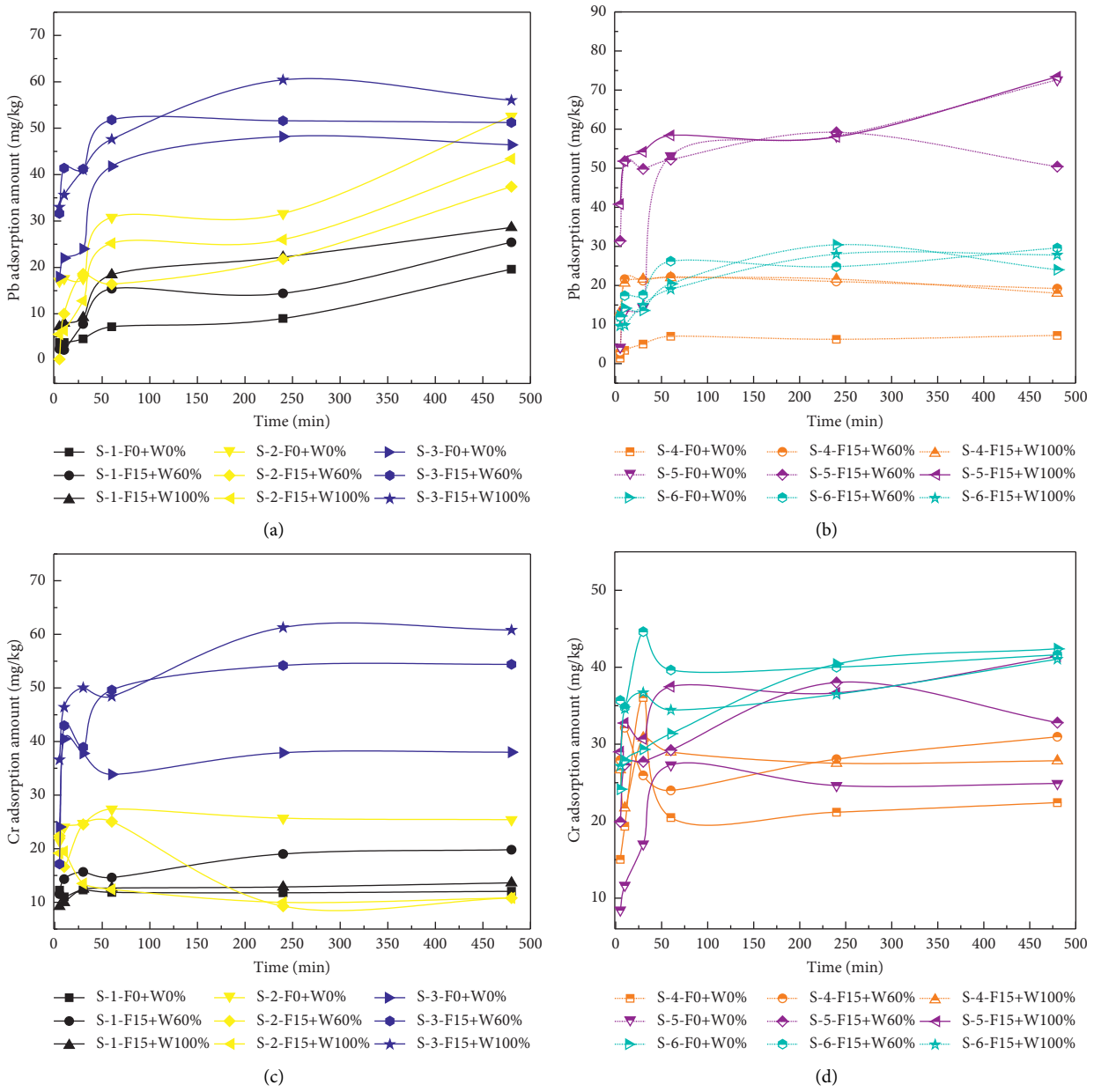

Figure 3: Adsorption amount of the Pb and Cr as a function of stirring time. F0 and F15 refer to the number of freeze-thaw cycles (0 and 15 cycles), and W0%, W60%, and W100% refer to water ratio of field capacity (0%, 60%, and 100% water ratio).

unfrozen in S-3, 0.14 h for soil under 15 FTCs and 60% water content, and 0.25 h for soil under 15 FTCs and 100% water content. The patterns of Cr adsorption in soil of S-6 under different conditions were similar to the soils in S-4. These results demonstrated that the kinetics of Pb adsorption under FT-treatments were more rapid than the unfrozen soils. Conversely, equilibrium of adsorption of Cr ions was achieved after 0.56 h for soil under unfrozen in S-3, 1.23 h for soil under 15 FTCs and 60% water content, and 0.71 h for soil under 15 FTCs and 100% water content. The results indicated that most Cr adsorption patterns on soils under freeze-thaw conditions were rapid than unfrozen conditions.

According to Table 4, parts of data could not be fitted using the pseudo-first-order model and Weber–Morris model. R^2 for the Pb pseudo-first-order model ranged from 0.001 to 0.91. R^2 for the Cr Weber–Morris model ranged from 0.02 to 0.95. Most of the R^2 data of the pseudo-first-order model and Weber–Morris model were lower than 0.8, the correlations were weak in most soil samplings under different conditions. For the control with no FT-treatment, the Q_e value was lower at 37.63 ± 1.84 in soils of S-3, while the Q_e values for the FT-treatments (F15 + W60% and F15 + W100%) were higher at 50.45 ± 3.93 and 55.03 ± 2.98, respectively. The soils in S-4, S-5, and S-6 were similar to the soils of S-3 under the same FT-treatments. The results indicated that most Cr adsorption amounts on soils under freeze-thaw conditions were higher than unfrozen conditions.

Table 3: Pseudo-first-order and Weber–Morris parameters of Pb in soils under different freeze-thaw conditions.

Site area	FTCs	Pb (pseudo-first-order kinetics)			Pb (Weber–Morris)		
		Q_e	k	r^2	C	k	r^2
S-1	F0 + W0%	22.95 ± 13.13	0.19 ± 0.21	0.72	1.13 ± 1.49355	5.69 ± 0.98	0.89
	F15 + W60%	20.46 ± 2.96	1.08 ± 0.49	0.84	1.28 ± 2.49	8.27 ± 1.64	0.86
	F15 + W100%	25.41 ± 2.76	1.27 ± 0.43	0.84	5.36 ± 1.75	8.48 ± 1.15	0.93
S-2	F0 + W0%	41.05 ± 7.4	1.69 ± 0.99	0.52	12.11 ± 3.43	13.07 ± 2.26	0.89
	F15 + W60%	28.35 ± 5.17	1.42048 ± 0.82	0.72	3.69 ± 3.71	11.37 ± 2.45	0.84
	F15 + W100%	35.53 ± 4.69	1.05 ± 0.43	0.85	3.29 ± 3.37	13.77 ± 2.22	0.90
S-3	F0 + W0%	45.79 ± 4.73	2.55 ± 0.95	0.76	19.26 ± 5.02	11.72 ± 3.31	0.75
	F15 + W60%	48.96 ± 2.22	11.84 ± 2.66	0.76	37.38 ± 4.05	6.15 ± 2.67	0.56
	F15 + W100%	51.23 ± 4.15	9.22 ± 3.39	0.56	33.61 ± 3.59	9.94 ± 2.37	0.81
S-4	F0 + W0%	6.78 ± 0.35	3.34 ± 0.66	0.94	2.95 ± 1.10	1.72 ± 0.73	0.58
	F15 + W60%	21.35 ± 2.53	7.49 ± 3.86	0.65	14.97 ± 5.27	2.53 ± 3.48	0.11
	F15 + W100%	21.00 ± 0.95	13.72 ± 3.32	0.74	18.88 ± 2.54	0.51 ± 1.68	0.02
S-5	F0 + W0%	67.23 ± 7.16	1.00 ± 0.33	0.91	4.51 ± 8.82	26.12 ± 5.82	0.83
	F15 + W60%	53.46 ± 2.37	12.57 ± 2.84	0.78	43.57 ± 6.10	4.61 ± 4.03	0.24
	F15 + W100%	60.90 ± 3.66	12.54 ± 3.83	0.61	44.71 ± 3.62	9.44 ± 2.39	0.79
S-6	F0 + W0%	23.95 ± 3.45	4.01 ± 2.32	0.46	12.03 ± 3.03	5.88 ± 2.00	0.68
	F15 + W60%	25.21 ± 2.19	6.34 ± 2.34	0.69	14.38 ± 2.53	5.70 ± 1.67	0.74
	F15 + W100%	26.94 ± 2.60	1.79 ± 0.57	0.83	8.76 ± 2.00	7.82 ± 1.32	0.89

Note. F0 and F15 refer to the number of freeze-thaw cycles (0 and 15 cycles), and W0%, W60%, and W100% refer to water ratio of field capacity (0%, 60%, and 100% water ratio).

Table 4: Pseudo-first-order and Weber–Morris parameters of Cr in soils under different freeze-thaw conditions.

Site area	FTCs	Cr (pseudo-first-order kinetics)			Cr (Weber–Morris)		
		Q_e	k	r^2	C	k	r^2
S-1	F0 + W0%	—	—	—	11.77 ± 0.35	0.06 ± 0.23	0.02
	F15 + W60%	17.21 ± 1.10	12.19 ± 3.92	0.59	12.34692 ± 0.87042	2.87 ± 0.57	0.86
	F15 + W100%	12.75 ± 0.44	12.71 ± 2.27	0.79	10.08 ± 0.78	1.41 ± 0.52	0.64
S-2	F0 + W0%	25.64 ± 0.51	20.82 ± 3.08	0.76	23.58 ± 1.30	0.97 ± 0.86	0.24
	F15 + W60%	—	—	—	24.39 ± 3.45	−5.21 ± 2.28	0.56
	F15 + W100%	—	—	—	18.26 ± 1.81	−3.35 ± 1.19	0.66
S-3	F0 + W0%	37.63 ± 1.84	15.42 ± 4.33	0.67	32.54 ± 4.10	2.32 ± 2.71	0.15
	F15 + W60%	50.45 ± 3.93	7.16 ± 2.41	0.77217	30.47 ± 7.16	10.28 ± 4.73	0.54
	F15 + W100%	55.03 ± 2.98	12.31 ± 3.35	0.67056	40.54 ± 3.14	8.33 ± 2.07	0.80
S-4	F0 + W0%	24.80 ± 3.26	10.75 ± 6.74	0.33883	—	—	—
	F15 + W60%	28.20 ± 1.52	59.07 ± 220.48	0.001	27.34 ± 2.23	0.68 ± 1.47	0.05
	F15 + W100%	27.50 ± 1.55	35.73 ± 32.36	0.0172	0.78 ± 1.47	26.30 ± 2.23	0.06
S-5	F0 + W0%	25.21 ± 1.60	3.36 ± 0.82	0.91004	12.08 ± 3.98	5.72 ± 2.63	0.54
	F15 + W60%	32.00 ± 1.96	11.48 ± 3.45	0.66061	23.50 ± 2.86	4.69 ± 1.89	0.60
	F15 + W100%	36.29 ± 1.91	18.04 ± 6.11	0.42226	29.72 ± 1.65	4.10 ± 1.09	0.77
S-6	F0 + W0%	35.72 ± 2.92	11.36 ± 4.52	0.48214	24.06 ± 1.18	7.06 ± 0.78	0.95311
	F15 + W60%	40.61 ± 1.56	22.07 ± 6.64	0.35317	37.35 ± 2.46	1.68 ± 1.62	0.21163
	F15 + W100%	37.18 ± 1.17	15.77 ± 2.89	0.77708	30.81 ± 2.16	3.52 ± 1.42	0.60374

Note. "—" means that these data could not be fitted using the pseudo-first-order and Weber–Morris models. F0 and F15 refer to the number of freeze-thaw cycles (0 and 15 cycles), and W0%, W60%, and W100% refer to water ratio of field capacity (0%, 60%, and 100% water ratio).

4. Conclusions

Results showed that different FT-treatments and soil water content affected the lead and chromium immobilization process in the northeastern Qinghai-Tibet Plateau. The different soil adsorption trends of Pb and Cr responded more or less constantly with change in the water-soil ratios. The Pb and Cr adsorption amount mostly decreased with increasing the water-soil ratio, and the soil performance of Pb and Cr adsorption at the same water-soil ratios showed variation under different freeze-thaw conditions. The adsorption of Pb was higher for most FT-treatments compared to the control. The soil performance of Cr isothermal adsorption showed variation under different freeze-thaw conditions. Most electrostatic binding of Pb and Cr was stronger under unfrozen and freeze-thaw conditions than unfrozen conditions. Most Pb adsorption kinetics patterns of FT-treated soils were rapid than unfrozen conditions; the

Q_e values in soils of S-3, S-4, and S-6 under FT-treatments increased compared to the control with no FT-treatment. Most Cr adsorption kinetics patterns on soils under freeze-thaw conditions were rapid than unfrozen conditions, and most Cr adsorption amounts on soils under freeze-thaw conditions were higher than unfrozen conditions. These results implied that FT-treatment could change the soil adsorption and desorption patterns of Pb and Cr. Therefore, further studies are urgently needed to investigate the lead and chromium immobilization mechanism of heavy metals in soils during FTCs.

Data Availability

The data are available on request.

Conflicts of Interest

No potential conflicts of interest are reported by the authors.

Acknowledgments

This work was supported by Natural Science Foundation of Qinghai Province (2019-ZJ-909), Youth Innovation Team Project for Talent Introduction and Cultivation in Universities of Shandong Province, Two Hundred Talents Plan of Yantai, and Thousand Talents Plan of Qinghai Province.

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Hindawi
Journal of Chemistry
Volume 2021, Article ID 9589345, 12 pages
https://doi.org/10.1155/2021/9589345

Research Article

Protective Effect of Nano-Vitamin C on Infertility due to Oxidative Stress Induced by Lead and Arsenic in Male Rats

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Received 1 July 2021; Revised 13 August 2021; Accepted 16 September 2021; Published 30 September 2021

Academic Editor: Hangbiao Jin

Occupational and environmental exposure to heavy metals such as arsenic (As) and lead (Pb) by inducing oxidative damage may impair male fertility. However, there is a new view that shows that the nano form of vitamins such as vitamin C, which have antioxidant activity, can be effective in improving this disorder. Therefore, this study aimed to evaluate the effect of NVC (NVC) on reproductive toxicity caused by the combination of Pb and As on testicular histology, sperm morphology, oxidative stress parameters, and hormonal changes in male rats. In this experimental study, forty-two male Wistar rats were randomly divided into six groups: control, NVC (200 mg/kg), As (50 ppm sodium arsenate), Pb (500 ppm Pb acetate), As + NVC, and Pb + NVC. FSH, LH, and testosterone levels were measured in serum. The activity of glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT), carbonyl protein, malondialdehyde (MDA), and total antioxidant capacity (TAC) was measured in testis. Histological examination and sperm parameters were also evaluated. FSH, LH, and testosterone levels and sperm parameters significantly decreased, and levels of protein carbonyl, MDA, and DNA fragmentation increased in the As and Pb groups, while treatment with NVC could improve them. Histological evaluation and sperm parameters in As and Pb groups showed damage in the process of spermatogenesis and sperm parameters. The treatment with NVC could significantly improve these parameters. The activity of GPx, SOD, and CAT in testis decreased in As and Pb groups, while treatment with NVC could enhance them. It can be concluded that NVC by inhibiting oxidative damage and improving serum level of testosterone, LH, and FSH could overcome As- and Pb-induced reproductive dysfunction.

1. Introduction

Occupational and environmental exposure to heavy metals such as As and Pb has increased in recent decades due to the development of industry and can adversely affect many functional systems of the body, including the endocrine system and reproduction [1, 2]. Exposure to pesticides, working in industry, and contaminated food sources can be a reason for contamination with these metals [3]. Infertility is a medical and psychiatric disorder in which couples are unable to conceive generally during a year of unprotected sex. Infertility has been a concern of the World Health Organization (WHO) in recent years [4].

Various mechanisms, including cell dysfunction, impairment of signaling pathways, oxidative stress, apoptosis, inflammation, and changes in endocrine function, are involved in harmful reproductive effects caused by heavy metals [5]. As and Pb bind to the thiol groups of protein and disrupt their function. In mitochondria, it depletes energy [6]. It can also cause the secretion of plasma glucocorticoids and suppress the secretion of gonadotropins [7, 8]. Oxidative stress can induce the expression of Bax and Bcl-2 genes. Oxidative stress, apoptosis, and cellular toxicity induced by As and Pb can cause male infertility. Therefore, antioxidant compounds can reduce the toxicity of spermatogenesis by decreasing the expression of apoptosis-inducing genes [7, 9].

As and Pb are nonessential metals that cause biochemical, physiological, and behavioral impairment in cells and organs. Studies have also shown that As and Pb poisoning is mediated by dysfunction of the pituitary-hypothalamic axis and the function of interstitial and Sertoli cells [10, 11]. They showed that Pb and As poisoning disrupts the reproductive system by altering the serum content of LH, FSH, and testosterone. Inflammation and oxidative damage are believed to play an important role in reproductive disorders caused by As and Pb [7, 11]. In addition, other studies have identified oxidative damage and inflammation as one of the basic mechanisms of infertility in men [12–15]. Therefore, it is assumed that inhibiting oxidative damage and inflammation by antioxidant and anti-inflammatory agents such as micronutrients and vitamins is a valuable and uncomplicated treatment strategy [16].

Vitamins are essential nutrients that can be easily obtained from the diet. Studies have shown that deficiency of vitamins C, B1, and B6 plays a role in the complications of cadmium and Pb poisoning, while supplementation of these vitamins has a significant protective role against the damage caused by heavy metal poisoning [17]. Vitamins C and E are natural nonenzymatic antioxidants that can scavenge free radicals and effectively inhibit lipid peroxidation. In addition, the protective effects of vitamins C on the liver, kidneys, brain, and testes against oxidative damage caused by exposure to Pb have been well demonstrated [18–20]. Vitamin C exerted nonenzymatic antioxidant function by destroying free radicals and trapping them in the aqueous phase to protect the biological membrane [21, 22]. However, there is a report that high doses of vitamin C have been suggested to cause oxidative stress and cell death [23]. Despite its widespread use as a potent antioxidant in the field of medicine, its use is limited. The biggest challenge in using vitamin C is maintaining its stability in the body and getting it to the active site [24]. At present, oxygen pressure control during formulation and storage and maintenance of proper temperature and pH are used for this purpose [18, 25]. In addition, the use of vitamin C derivatives and the use of new drug delivery systems such as microencapsulation and nanotechnology are new solutions that have attracted special attention in the last decade [25]. Today, nanotechnology is advancing rapidly in the world. Special attention has been paid to its applications in industry, catalysts, sensors, cancer therapies, and free radical scavengers [23]. Therefore, the administration of proper doses and particle size of vitamin C can possibly exert appropriate biological effects. Due to the above explanations and the lack of a similar study, the aim of this study was to investigate the effects of nanoparticles of vitamin C (NVC) on reproductive damage caused by exposure to Pb and As.

2. Materials and Methods

2.1. Animals. The present study was an experimental-interventional study that was performed on forty-two adult male Wistar rats (weight range: 200–230 g and age range: 7–8 weeks) purchased from Pasteur Institute of Iran. All stages of this study were performed based on the guide for the care and use of laboratory animals approved by Kurdistan University of Medical Sciences with the reference no. IR.MUK.-REC.1399.6014. Animals were kept at $20 \pm 2^{\circ}C$ and 12 hours light: 12 hours dark-light cycle and had free access to chow and water. After one week of familiarization, the animals were randomly divided into controlled and treated groups.

2.2. Chemicals. Sodium arsenate, lead acetate, and vitamin C nanoparticles were purchased from Sigma-Aldrich (Germany). Sodium arsenate (dose) and lead acetate (dose) were added into drinking water; vitamin C nanoparticles were also prepared in a 10% oral solution at a dose of 200 mg/kg.

2.3. Acute Toxicity Study. An acute toxicity study was performed to determine the lethal dose (LD50) of vitamin C nanoparticles (50–1000 mg/kg). Twenty-five adult male rats (200–230 g) were randomly divided into five groups. The groups received (P.O.) 50, 100, 200, 400, and 1000 mg/kg body weight of vitamin C nanoparticles, respectively. They were monitored for abnormal symptoms and mortality for the first 24 hours after injection. After this period, this process lasted up to 14 days for chronic complications. In the study, acute oral poisoning with different doses ranging from 50 to 1000 mg/kg was investigated. No mortality was observed at the maximum dose (1000 mg/kg). Belodarased on this and earlier studies that have used an oral dose of 200 mg/kg of vitamin C (Jelodar et al., 2013, [24]), we chose a similar dose, 200 mg/kg, to continue the study [26].

2.4. Experimental Design. Animals were randomly divided into six groups of seven to evaluate the protective effects of vitamin C nanoparticles [27].

Control group (C): animals received only water and chow.

As group (As): animals received 50 ppm sodium arsenite in drinking water for 30 days.

Pb group (Pb): animals received 500 ppm lead acetate in drinking water for 30 days.

NVC (NVC): animals received nano-vitamin C (200 mg/kg/day) which was given by gavage for 30 days.

As + NVC group: animals received 50 ppm sodium arsenate via drinking water and 200 mg/kg nano-vitamin C (200 mg/kg/day) daily by gavage.

Pb + NVC group: animals received 500 ppm lead acetate via drinking water and 200 mg/kg nano-vitamin C (200 mg/kg/day) daily by gavage.

The duration of the study was 30 days to evaluate the effectiveness of nanoparticles of vitamin C in subacute toxicity dose in exposure to Pb and As on the reproductive system [28–31].

2.5. Sampling and Measurement of Hormonal Variables. The weight of the animals was measured before and at the end of the study period. After the last day of treatment,

animals were anesthetized by intraperitoneal injection of ketamine (100 mg/kg) and xylazine (10 mg/kg). Blood samples were taken from the heart, and serum was separated by centrifugation (3000 rpm, 10 min) and used to measure hormonal parameters. Serum concentrations of LH, FSH, and testosterone were measured using the radioimmunoassay (RIA) method. The hormonal diagnostic kits (Catalog# KA2240) used in this study were purchased from Pars Azmoun Co., Tehran, Iran. The hormonal kits included standard solutions of radioactive iodine, antibodies, and wash buffer. The basis of this method was the competition between the antigens in the assay sample for binding to the antibody, which used radioactive antibodies and a buffer with less than 10% free sodium [32, 33].

2.6. Preparation of Tissue Samples and Measurement of Oxidative Damage Parameters. The testes were immediately removed after blood sampling. The weight of the right and left testes was measured. After washing the testis with cold saline and placing it inside the microtube, the testicular samples were frozen in liquid nitrogen, and they were then stored at −80°C. At the time of measurement, testicular tissue was manually homogenized in phosphate buffer (0.1 M, pH: 7.4), and debris was removed by centrifugation at 3000g for 10 min. After centrifugation (2750 rpm, 10 min), the resulting supernatant was used for biochemical testing [34]. Total antioxidant capacity (TAC) was measured by the ferric-reducing antioxidant power (FRAP) method. In this method, the ability of plasma to reduce ferric ions in the presence of free radicals was measured. At acidic pH, when the FeIII-TPTZ complex was reduced to FeII, it produced a blue dye that had the highest light absorption at 593 nm. TAC values were measured using a standard solution of $FeSO_4$ that has been used for calibration of the analysis. The outcomes are expressed in μmol/L $FeSO_4$ [35]. The enzyme glutathione peroxidase (GSH-Px) catalyzes the oxidation of glutathione by the cumene hydroperoxide. The oxidized glutathione is then converted back to regenerative glutathione in the presence of the enzyme's glutathione reductase and NADPH. In this experiment, the $NADP^+$ obtained at 340 nm was measured. The basis of superoxide dismutase (SOD) activity was to inhibit the conversion of superoxide to H_2O_2 and O_2, which was measured at a wavelength of 505 nm as described by a previous study [36]. Catalase activity was measured by the Aebi method based on H_2O_2 decomposition at 240 nm [36, 37]. Malondialdehyde (MDA) measurement was determined based on the reaction with thiobarbituric acid (TBA) using spectrophotometry at 532 nm [38]. Testicular protein carbonyl content was measured by the Levine method. In this method, reagents 2,4-dinitrophenylhydrazine re-shift with the carbonyl groups present in the proteins to form a yellow complex whose color intensity was spectrophotometric at 380 nm [37]. Total protein was measured by the Bradford method [39].

2.7. Measurement of Sperm Parameters. According to the criteria of the World Health Organization (WHO), the sperm analysis was performed as follows. A hemocytometer slide was used to assess sperm count. Only sperm cells with head, middle, and tail were counted using a light microscope with a magnification of x40. Counting was done twice for each sample, and its mean was recorded. Results were expressed as the number of sperm per ml of semen [40]. To evaluate the motility of sperm, 10 μl of each sample was placed in the center of the Makler counting chamber, and it was observed and counted by a light microscope with a magnification of ×40. In this study, 200 sperm cells were counted, and the percentage of sperm motility was determined according to the guidelines of WHO. The evaluation of sperm viability was performed using eosin-nigrosin staining. First, eosin solution (1%, Merck) was added to the sperm solution (2 : 1 ratio), and after 30 s, the nigrosin solution (10%, Merck) was added to it. 10 μl of the new solution was smeared on a slide and then air-dried. Finally, sperm viability was assayed under a light microscope (100× magnification) [41].

2.8. DNA Fragmentation Assessment. Sperm DNA fragmentation was assessed by a sperm DNA fragmentation kit (Avicenna, Iran) using the sperm chromatin dispersion (SCD) method according to the manufacturer's instruction. In brief, the sperm cells were washed twice with the PBS buffer, and then a suspension containing 15–20 million sperm solutions was prepared. After that, spermatozoa were immersed in agarose microgel and smeared on the slide. In addition, denaturation by acid and lysis solution, dehydration, and staining with Diff-Quick were done. Sperm cells with large halos (that were similar or larger than the diameter of sperm's head) and sperms with medium-sized halos (halo greater than 1 : 3 of the smallest diameter of the sperm's head and less than the smallest diameter) were defined as spermatozoa having no fragmentation [42, 43].

2.9. Histological Evaluation and Study of Spermatogenesis. The right testis was wholly removed and fixed in a 10% formalin solution. It was then embedded in paraffin after a week, and sections with a thickness of 5 microns were prepared and then stained with hematoxylin and eosin. Histological features were examined with a light microscope (BX-51, Olympus Corporation, Tokyo, Japan) with the same magnification (40x). Moreover, spermatogonia, spermatocytes, spermatids, Leydig cells, and Sertoli cells were counted in 5 fields for each section under a light microscope [44]. Seminiferous tubule diameter, lumen diameter, and epithelial thickness were measured in different groups using a counting probe and Motic Images software [44, 45]. The percentage of tubule vacuolization, tubule with desquamated cells, and pyknotic cells was also measured using ZILOS-tk, version 5.9. The spermatogenesis and sperm maturation process was analyzed by histological examination of seminiferous tubules. Fifty seminiferous tubules were cross-sectionally evaluated and scored on a scale of one to ten based on defined criteria. Scoring included the following: 1—there are no germ cells and Sertoli cells; 2—there are no germ cells; 3—there are only spermatogonia; 4—there are only a few spermatocytes; 5—there are many spermatocytes but no spermatozoa or spermatids; 6—there are only a few

spermatids; 7—there are many spermatids but no spermatozoa; 8—there are only a few spermatozoa; 9—there are many spermatozoa but disorganized spermatogenesis; and 10—spermatogenesis and tubules are observed completely and perfectly [46].

2.10. Statistical Analysis. All data are presented in mean ± SEM. The normality of the data was evaluated by the Kolmogorov-Smirnov test. One-way ANOVA followed by Tukey's post hoc test were performed to analyze the parametric data. All graphs and statistical analyses were performed by GraphPad Prism version 7 software (GraphPad Software, San Diego, California, USA).

3. Results

3.1. Body Weight and Testis Index. The As and Pb groups had lower testis weight as compared to the other groups. Moreover, the testis index in As and Pb groups significantly decreased as compared to other groups (Table 1) ($p = 0.002$).

3.2. Serum Levels of Reproductive Hormones. The highest level of FSH was seen in the NVC group, and the lowest level was seen in the Pb and As groups (Table 2). Nonetheless, in the As and Pb groups which received NVC, the level of FSH, LH, and testosterone increased compared with the As and Pb groups. In some cases, these differences were not significant ($p = 0.035$). Serum testosterone concentration in the NVC and control groups was the highest level in comparison to the other groups ($p = 0.007$).

3.3. Sperm Parameters. Parameters related to the morphology of epididymal sperm cells in the different groups are presented in Table 3. Sperm concentration in control and NVC groups was significantly higher than that of the Pb and As groups ($p = 0.035$). The percentage of survival of sperm in control and NVC groups was significantly higher than that in the Pb and As groups ($p < 0.05$). The Pb and As groups had the highest percentage of dead sperm compared to other groups. However, these parameters improved in the As and Pb groups which received the NVC as compared to the As and Pb groups, and this difference was not substantial. The highest amount of motility percentage was observed in the NVC group, and the lowest level was seen in the Pb group. The highest percentage of DNA fragmentation was observed in the As group, which had a significant difference from the NVC group. DNA fragmentation level significantly improved in the As + NVC and Pb + NVC groups as compared with that in the As and Pb groups ($p < 0.05$).

3.4. Study of Spermatogenesis. The number of spermatogonia significantly decreased in the As and Pb groups compared to the control group ($p = 0.046$). The mean number of spermatocytes significantly decreased in the As treatment compared to the control and NVC groups ($p < 0.05$). These parameters significantly improved in the As and Pb groups receiving NVC compared to the As and Pb groups. The maximum number of spermatid cells was seen in the NVC group, which was significantly different from the As and Pb groups ($p < 0.001$). The decrease in the number of Leydig cells and Sertoli cells in the As and Pb groups was significantly different from those in control and NVC groups ($p < 0.01$) (Table 4).

3.5. Histological and Testicular Structure. The diameter of the seminiferous tubules in the As and Pb groups showed a significant difference compared to the control group. There is no significant difference between the control group and the NVC group. The highest epithelial thickness of seminiferous tubules was observed in the NVC group, and the lowest was in the As group. There was a significant difference between them ($p < 0.01$). The percentage of vacuolation tubes significantly increased in the As and Pb groups in comparison with the control group ($p < 0.001$), while administration of NVC in As + NVC and Pb + NVC groups could significantly improve this effect. The lowest level of tubule with desquamated cells was observed in the NVC group, and the highest level was observed in the As group. This revealed a significant difference between these groups ($p < 0.01$). The minimum level of pyknotic cells was observed in the control group, and the maximum level was in the As group describing a significant difference between these groups ($p < 0.01$). The highest Johnsen score was observed in the group which received NVC, and the lowest was in the As and Pb groups. There was a significant difference between the control group and the As and Pb groups according to this parameter. On the other hand, administration of NVC in the As + NVC and Pb + NVC groups could significantly improve this effect (Table 5 and Figure 1).

3.6. Oxidative Stress Parameters. The highest level of GPx was observed in the control and NVC groups, and the lowest was in the Pb group. In the As and Pb groups treated with NVC, the level of this enzyme had significant with the control group ($p = 0.038$). The highest activity of SOD was in the NVC group, and the lowest was in the As group. The activity of this enzyme in the As and Pb groups treated with NVC significantly improved as compared with that in the As and Pb groups ($p = 0.032$). The highest CAT activity was in the NVC group, and the lowest level was in the Pb group. There was a significant difference among the NVC group with other groups except for the control group ($p < 0.05$). The level of MDA considerably increased in As and Pb groups compared to the control and NVC groups, while the level of this parameter significantly decreased in the As and Pb groups treated with nano-vitamin C in comparison with the As and Pb groups ($p < 0.05$). Testicular carbonyl protein concentration in As and Pb groups significantly increased as compared to the control group. There was a significant difference among the carbonyl protein concentration of the control group with other groups except for NVC. The amount of carbonyl protein in the As and Pb groups treated with NVC showed a major decrease in comparison with the As and Pb groups ($p < 0.05$). The level of TAC revealed a

Table 1: Effect of NVC on body weight and testicular absolute and relative weights of As- and Pb-exposed rats.

Groups	Initial body weight (g)	Final body weight (g)	Body weight change	Right testis weight (g)	Left testis weight (g)	Testis index (%)
C	214.0 ± 3.60	239.8 ± 6.02	25.8 ± 2.42	1.08 ± 0.063	1.11 ± 0.079	0.45 ± 0.011
As	211.3 ± 3.20	215.4 ± 7.60 a*	4.1 ± 4.40 a*	0.878 ± 0.02	0.84 ± 0.031 a*, d*	0.39 ± 0.335 a*
Pb	218.8 ± 2.00	221.5 ± 4.40 a*	2.7 ± 2.41 a*	0.928 ± 0.023	0.91 ± 0.021 a*	0.41 ± 0.026 a*
NVC	213.4 ± 1.91	236.11 ± 3.40 b*	22.71 ± 1.52 b**, c**	1.10 ± 0.049 b*	1.08 ± 0.045 b*	0.46 ± 0.0.013 b*, c*
As + NVC	217.4 ± 4.5	231.6 ± 2.11 b*	14.23 ± 1.98b*, c*	0.94 ± 0.059	1.03 ± 0.046	0.42 ± 0.015a*, d*
Pb + NVC	213.4 ± 2.50	229.11 ± 6.21	15.71 ± 3.71b*, c*	1.021 ± 0.63	1.04 ± 0.048	0.44 ± 0.0.054 b*

All values are presented as mean ± SEM. * p value <0.05; ** p value <0.01. a, b, c, and d, respectively, are compared with C, As, Pb, and NVC groups. Final body weight and body weight changes significantly increased in the As + NVC and Pb + NVC groups compared to Pb and As groups ($p = 0.015$).

Table 2: Comparison of changes in the serum concentration of hormones in the studied animals.

Groups	FSH (ng/mL)	LH (ng/mL)	Testosterone (ng/mL)
C	2.11 ± 0.079	6.14 ± 0.268	5.55 ± 0.099
As	1.39 ± 0.136 a*	4.95 ± 0.254 a**	2.42 ± 0.064 a**
Pb	1.3 ± 0.071 a*	5.14 ± 0.232	2.20 ± 0.130 a***
NVC	2.42 ± 0.141 b**, c*	6.41 ± 0.223 b*	5.50 ± 0.219 b**, c**
As + NVC	1.4 ± 0.752 *a, d**	5.27 ± 0.120 b*, d*	2.80 ± 0.332 a*, b*, d
Pb + NVC	1.41 ± 0.730 a*, b*, d**	5.31 ± 0.201 b*, d*	2.21 ± 0.075 a***, d**, e*

All values are presented as mean ± SEM. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. a, b, c, and d are compared with C, As, Pb, and NVC groups, respectively.

Table 3: Effect of NVC on sperm parameters of As- and Pb-exposed rats.

Groups	Sperm concentration ($\times 10^6$/ml)	% vitality	% dead sperm	% motility	% DNA fragmentation
C	345.47 ± 6.217	85.00 ± 0.925	15.00 ± 0.925	63.14 ± 1.223	55.14 ± 0.508
As	234.42 ± 22.229 a*	44.57 ± 0.611 a**	55.42 ± 0.611 a***	30.71 ± 0.420 a*	75.13 ± 0.594 a**
Pb	214.57 ± 2.827 a**	44.28 ± 0.993 a**	55.43 ± 1.020 a***	30.00 ± 0.654 a*	69.00 ± 2.370 a**, b*
NVC	337.57 ± 20.496 b*, c*	81.42 ± 6.105 b****, c**	18.57 ± 6.105 c b**	69.28 ± 6.398 b**, c*	44.42 ± 0.895 a*, b**, c*
As + NVC	287.85 ± 15.615 c*	54.14 ± 0.857 a*, d*	45.85 ± 0.857 a*, d*	43.14 ± 0.769 a*, b*, c*, d*	55.28 ± 0.565 b*, c*, d***
Pb + NVC	278.42 ± 3.198 a*, c*	54.87 ± 0.828 a*, d*	44.28 ± 0.778 a*, b*, c*, d*	44.85 ± 0.508 a*, b*, c*, d*	56.00 ± 0.975 b*, c*, d***

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. a, b, c, and d are compared with C, As, Pb, and NVC groups, respectively.

Table 4: Effect of NVC on spermatogenic cell count in the testis of As- and Pb-exposed rats.

Groups	Spermatogonium	Spermatocyte	Spermatid	Leydig cell	Sertoli cell
C	48.00 ± 0.816	56.42 ± 0.649	59.00 ± 2.115	8.28 ± 0.420	6.13 ± 0.404
As	32.14 ± 0.508 a*	35.57 ± 1.556 a**	45.43 ± 1.231 a***	3.57 ± 0.368 a***	3.00 ± 0.308 a***
Pb	32.42 ± 0.649 a*	40.42 ± 0.996 a**, b	48.28 ± 2.043 a**	3.85 ± 0.404 a***	3.28 ± 0.285 a***
NVC	45.42 ± 1.986 b*, c*	56.85 ± 0.857 b*, c*	62.57 ± 1.324 b***, c**	7.71 ± 0.865 b**, c**	5.85 ± 0.341 b**, c**
As + NVC	40.25 ± 1.426 a*, b**, c**	46.57 ± 1.172 a*, b**, c*	52.42 ± 0.719 a*, b**, d**	5.57 ± 0.368 a**	3.14 ± 0.340 a***, d**
Pb + NVC	37.71 ± 1.209 a**, b*, c*, e*	43.00 ± 0.816 a**, b*	52.57 ± 0.649 a*, b**, d*	5.85 ± 0.508 a**, b*	3.00 ± 0.308 a***, d**

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. a, b, c, d, and e are compared with C, As, Pb, NVC, and As + NVC groups, respectively.

decrease in As and Pb groups as compared to the control group ($p < 0.01$). Moreover, the amount of TAC in the As and Pb groups treated with nano-vitamin C significantly increased as compared to those in the As and Pb groups ($p = 0.043$) (Figure 2).

4. Discussion

4.1. Body and Testicular Weight Changes. Biological damage caused by environmental pollutants such as heavy metals such as Pb and As is a major concern of human societies that has been addressed in recent decades. Among these damages, reproductive activity defects are more important because of their relationship with the survival of the human race, and efforts to prevent and control them have always been a priority. Antioxidant defense mechanisms that are disrupted by these metals after oxidative damage are one of the main causes of reproductive failure due to exposure to these metals [47]. Our results showed that exposure to Pb and As, in addition to reproductive disorders, reduced body weight and testicular index, which was consistent with the results of other researchers. However, these results are

Table 5: Histological results of testicular tissue and spermatic tubule in different groups.

Groups	Seminiferous tubule diameter (μM)	Tubule vacuolization (%)	Seminiferous epithelium thickness (μM)	Lumen diameter (μM)	Pyknotic cell (%)	Tubule with desquamated cells (%)	Johnsen score
C	473.28 ± 1.82 2	4.71 ± 0.420	111.14 ± 1.682	230.57 ± 1.586	1.71 ± 0.285	4.71 ± 0.565	10.03 ± 0.047
As	406.71 ± 413 a**	31.37 ± 2.20 a***	97.85 ± 0.857	217.57 ± 1.937 a*	11.57 ± 1.50 a***	31.57 ± 2.67 a***	6.77 ± 0.48a***
Pb	397.14 ± 6.745 a***	30.71 ± 2.337 a***	98.83 ± 1.10	218.58 ± 2.102 a*	12.28 ± 1.53 a***	30.51 ± 1.643 a***	7.35 ± 0.034a***
NVC	439.57 ± 2.213 b*, c**	5.57 ± 0.649 b***, c***	126.42 ± 14.272 b**, c**	231.42 ± 1.445 b*, c*	3.85 ± 0.853 b*, c**	4.60 ± 0.565 b***, c**	9.85 ± 0.024b*
As + NVC	409.28 ± 5.139 a**, d*	19.57 ± 1.269 a**, b*, c**, d**	104.85 ± 1.335	221.28 ± 1.106 a*, d*	8.42 ± 1.19 a**	27.85 ± 1.994 a**, **c, d**	8.69 ± 0.028a*, c*
Pb + NVC	402.00 ± 4.654 a**, d*	17.42 ± 1.296 a**, b, c**, d**	105.57 ± 0.812	221.71 ± 2.542 a*, d*	7.78 ± 1.01 a**, d*	26.00 ± 1.327 a**, c**, d**	8.12 ± 0.094a*, c*

All values are presented as mean ± SEM. * p value <0.05; ** pp value <0.01; *** p value <0.001. a, b, c, and d, respectively, are compared with C, As, Pb, and NVC groups.

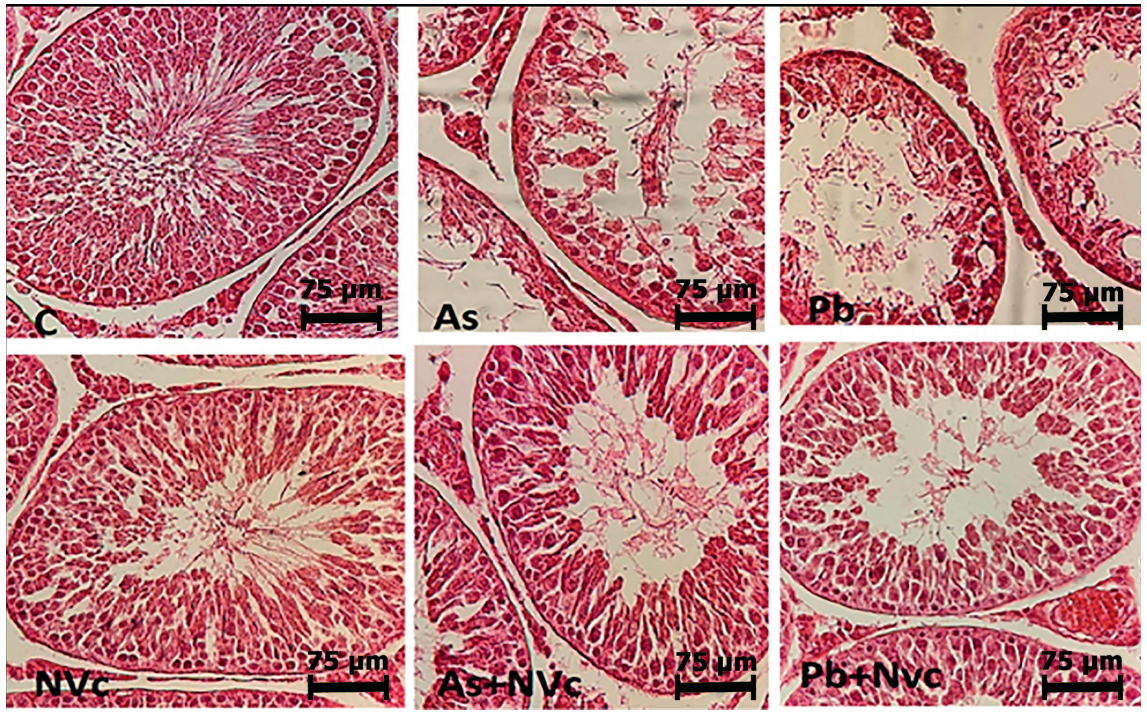

Figure 1: Histological sections of seminiferous tubules prepared from different groups. C: control group; different levels of spermatogenesis cells regularly and without changes in necrosis; normal thickness of seminiferous tubules. As: severe decrease in the mean of different cells in the process of spermatogenesis in seminiferous tubules, and decrease in the diameter of seminiferous tubules. Pb: reducing the number of the different cells in the process of spermatogenesis and reducing the diameter of the lumen and seminiferous tubules. NVC: maximum presence of the different spermatogenesis cells and no sign of cell necrosis. As + NVC and Pb + NVC: increasing the mean of the different cells of spermatogenesis process and increasing the diameter of seminiferous tubules and necrotic cells. H&E (×400). Scale bar: 75 μm.

significantly improved by the consumption of vitamin C nanoparticles. Gaskill et al. [48], Joseph et al. [49], and Bhatia et al. [50] also claimed that weight loss due to exposure to heavy metals could be due to the body's imbalance in food intake and absorption, the latter of which could be due to inhibition of enzymes involved in digestion and absorption of nutrients. Moreover, in Ezedom and Asagba et al. it was reported that prolonged exposure to heavy metals such as As could reduce the absorption of nutrients. Researchers have shown that cadmium and As can accumulate in various body tissues, including the liver, brain, kidneys, and testes, leading to the inhibition of oxidative enzymes, thus affecting several normal metabolic processes [37].

4.2. Hormonal Changes. Gonadotropins, testosterone, and their interaction with regulatory centers in the central nervous system play a very important role in controlling the function of the reproductive system. LH is a prerequisite for gonadal function, and FSH is responsible for spermatogenesis and normal testis activity [51]. Several possible mechanisms have been proposed for heavy metals and their damaging effects on the reproductive system, as well as changes in gonadotropin levels [52]. The results of this study indicate the disruptive role of As and Pb on the nerve centers that regulate the synthesis and release of FSH and LH. Pb as an endocrine disrupter can reduce the release of FSH and LH. Therefore, a decrease in LH concentration reduces the number and function of Leydig cells, secreting testosterone in groups treated with heavy metals. Moreover, oxidative damage and the decrease in FSH and LH concentration, in addition to cytological changes in testicular tissue, reduce cell lines from spermatogonium to spermatid and ultimately reduce sperm concentration [53], the results of which were well demonstrated in our study. These results showed that the concentrations of FSH, LH, and testosterone, which were reduced with As and Pb, could be increased by treatment with NVC. Karanth et al. showed that vitamin C leads to the release of LH and FSH from the pituitary gland; it increases the level of intracellular calcium ions and activates the release of these hormones [54].

4.3. Sperm Morphology. As a result of As intake, concentration, vitality, and motility in the sperm decreased significantly. ROS production as well as As binding to thiol groups in protein and chromatin of spermatozoa can cause significant changes in sperm dysfunction [55].

Pb reduces fertility by impairing the release of FSH, LH, and testosterone and leads to a decrease in sperm production and abnormal morphology. Vitamin C also increased sperm concentration, motility, and viability in a dose-dependent manner. In line with the results of this study, Okon and Uduak showed that vitamin C at a dose of 400 mg/kg could have a similar effect; they concluded that vitamin C has a strong effect on male reproductive function. Thus, the use of NVC at 200 mg/kg increases the levels of FSH, LH, and

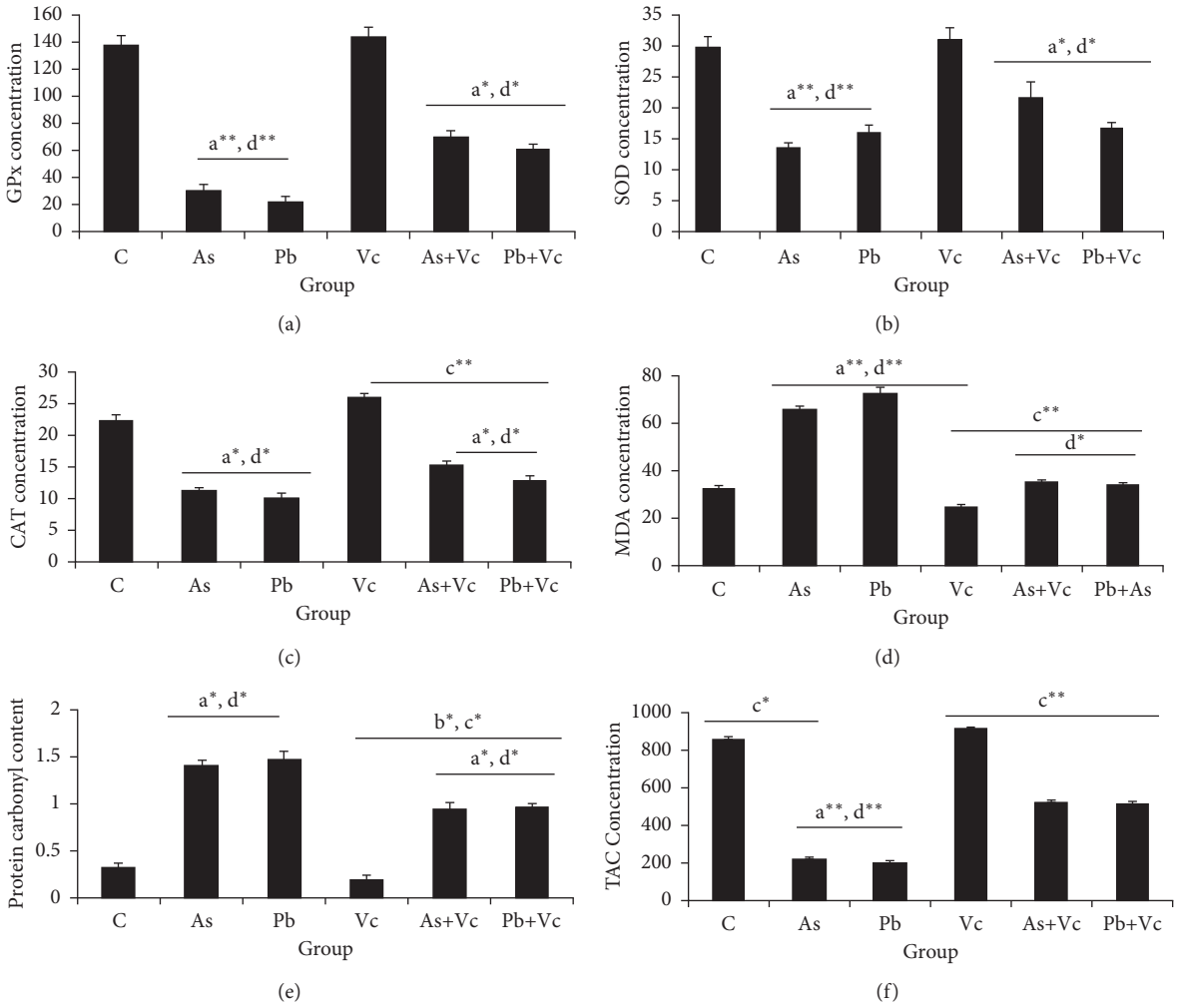

Figure 2: Mean ± SEM of testicular oxidative markers in different groups. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$. a, b, c, and d are compared with C, As, Pb, and NVC groups, respectively. (a) Glutathione peroxidase (GPx). (b) Superoxide dismutase (SOD). (c) Catalase (CAT). (d) Malondialdehyde (MDA). (e) Protein carbonyl content. (f) Total capacity antioxidant (TAC).

testosterone directly, which can subsequently control oxidative stress [56].

The effect of vitamin C on testosterone levels and sperm parameters in gentamicin intoxication was investigated by Rahayu et al. [57]. Their results, in agreement with our results, showed that NVC increased testosterone level and improved sperm parameters [57]. NVC could improve reproductive function by increasing the number of spermatozoa cells, sperm concentration, motility, and viability and neutralizing oxidative damage caused by As and Pb.

4.4. Spermatogenesis Changes. Pb also, directly and indirectly, affects interstitial and Sertoli cells in a reproductive function [28]. The results of the present study show the effects of cytotoxicity of As and Pb on sperm parameters. Testicular weight loss can be due to a decrease in the diameter of the seminiferous tubules, a decrease in the thickness of the germ membrane in the seminal vesicles, and an increase in the percentage of pyknotic cells that indicate apoptosis caused by exposure to Pb and As [33]. One of the main reasons for testicular weight loss and disruption of spermatogenesis is the formation of free radicals and reduced antioxidant capacity of testicular tissue. However, these changes were ameliorated by the antioxidant effects of NVC.

Damage that resulted from exposure to As and other heavy metals causes oxidative stress, inflammation, and apoptosis, which can be inhibited by antioxidant and anti-inflammatory compounds [33]. On the other hand, the possible accumulation and complications of As and Pb in testicular tissue induce free radicals and apoptosis in testes. Therefore, the spermatogenesis and testicular weight decreased. NVC improved body weight, gonadal hormones, and other changes in testicular tissue of As and Pb groups. It could be due to inhibiting oxidative stress, inflammation, and apoptosis [24, 58–60]. Our results, in line with previous reports, showed that administration of NVC might have improved testicular function in the As and Pb groups through its antioxidant effects [60]. El-Sayed et al. showed that vitamins C and E could improve the toxic effect of Pb on spermatogenic cell

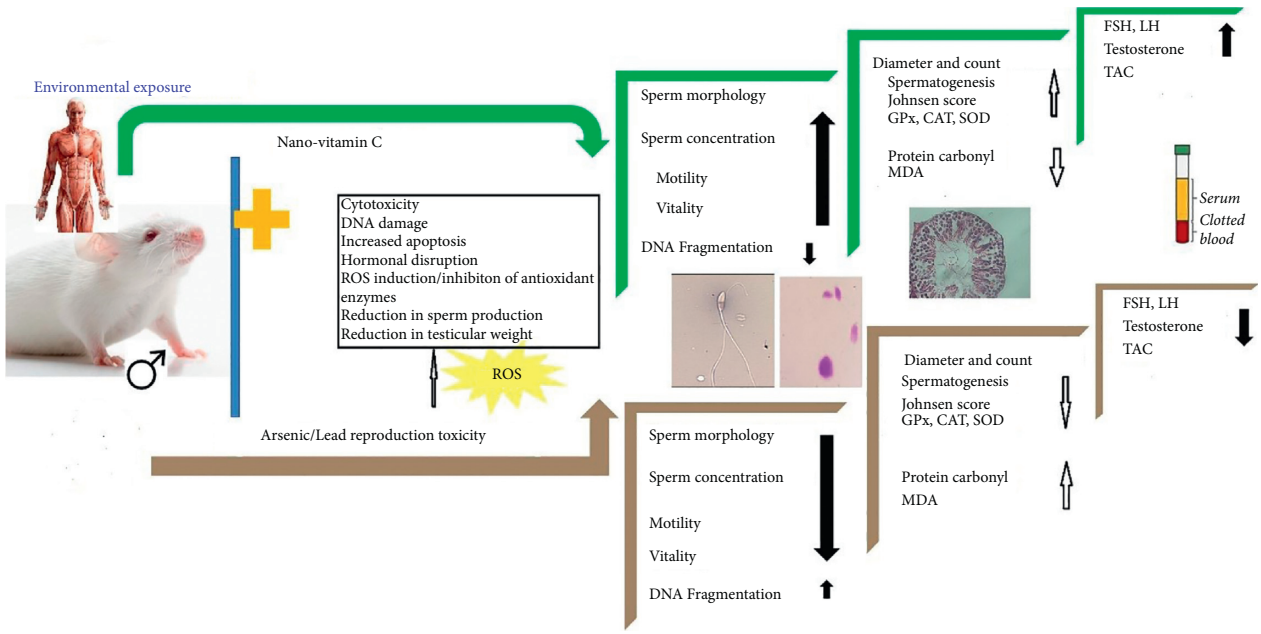

Figure 3: Diagram of the summary of the study results.

necrosis, spermatogenesis cells of tubules, edema, and interstitial hemorrhage [61].

In another study, the effect of vitamins C and E and nanoselenium on the weight and biochemical and serological parameters of ammonium-induced oxidative stress was investigated [62]. The results showed that vitamins C and E and nanoselenium increase weight, control biochemical changes, and strengthen the immune system [63]. In addition, vitamin C has been shown to reduce the accumulation of Pb in red blood cells, liver, and brain by increasing catalase levels [64]. NVC and vitamin E could also reduce inflammation and pain after surgery [65]. In another work, the antioxidant and anti-apoptotic effects of vitamin C in doxorubicin treatment showed that treatment with them reduced sperm DNA fragmentation and had protective effects on the reproductive system [66]. However, heavy metal damage may lead to oxidative stress, inflammation, and apoptosis, which induce the inhibition of antioxidant and anti-inflammatory compounds [67, 68].

On the other hand, oxidative stress increases the expression of Box and Bcl-2 and can cause apoptosis in Leydig and Sertoli cells. This condition damages their receptors (LHCGR and FSHR), leading to the formation of a negative feedback loop, which inactivates the hypothalamus to secrete GnRH. Therefore, oxidative stress can reduce the concentration of LH and FSH in Pb and As poisoning. Under these conditions, the use of antioxidants such as NVC improved reproductive performance. This is mainly achieved by reducing cellular oxidative damage and regulating this signaling pathway [53, 60].

5. Conclusions

Most male infertility can be attributed to oxidative damage due to various environmental and occupational exposures to environmental pollutants, waves, and heavy metals such as As and Pb. It can be concluded that, in addition to oxidative damage, a decrease in FSH and LH concentrations, changes in cytological testicular tissue, a decrease in cell lines from spermatogonium to spermatid, and finally a decrease in sperm parameters after exposure to As and Pb were observed in this study. Moreover, NVC improved the reproductive damage caused by exposure to Pb and As by increasing the concentration of antioxidant enzymes and reducing the level of oxidative damage parameters. In addition, NVC can increase the concentration of LH, FSH, and testosterone and improve sperm parameters and the process of spermatogenesis. The results of this study suggested that the use of antioxidant compounds containing vitamin C in the form of nanoparticles (possible to increase stability) can improve oxidative stress conditions in infertility, which can be caused by environmental pollutants in the ecosystem (Figure 3).

5.1. Study Limitations and Suggestions. According to the use of antioxidants such as vitamin C and E to treat infertility, the synthesis of NVC in the pharmaceutical market after comparative clinical studies with vitamin C is proposed to extend the results of the present study. Finding the exact toxic dose of NVC in vitro was one of the research limitations of this study, which is recommended to be considered in future studies. Moreover, investigation of the molecular path involved in oxidative damage and apoptosis is one of the limitations of the study which is recommended to complete the results.

Abbreviations

NVC: Nano-vitamin C
RIA: Radioimmunoassay
As: As group

FRAP: Ferric-reducing antioxidant power
Pb: Pb group
TBA: Thiobarbituric acid
TAC: Total antioxidant capacity
SOD: Superoxide dismutase
CAT: Catalase
GPx: Glutathione peroxidase
MDA: Malondialdehyde
ROS: Reactive oxygen species
WHO: World Health Organization
ALAD: δ-Aminolevulinic acid dehydratase.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Additional Points

Highlights. (i) Nonessential heavy metals such as As and Pb are toxic to the reproductive systems. (ii) Oxidative stress is one of the major causes of infertility. (iii) Like the first report, NVC increased male reproductive function against Pb and As toxicity.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

The authors would like to sincerely appreciate the Research Vice Chancellor at the Islamic Azad University, Sanandaj Branch, for their support in animal studies.

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Hindawi
Journal of Chemistry
Volume 2021, Article ID 7694157, 21 pages
https://doi.org/10.1155/2021/7694157

Review Article

Bioremediation of Chromium by Microorganisms and Its Mechanisms Related to Functional Groups

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Received 2 June 2021; Revised 21 June 2021; Accepted 22 June 2021; Published 1 July 2021

Academic Editor: Yifeng Zhang

Heavy metals generated mainly through many anthropogenic processes, and some natural processes have been a great environmental challenge and continued to be the concern of many researchers and environmental scientists. This is mainly due to their highest toxicity even at a minimum concentration as they are nonbiodegradable and can persist in the aquatic and terrestrial environments for long periods. Chromium ions, especially hexavalent ions (Cr(VI)) generated through the different industrial process such as tanneries, metallurgical, petroleum, refractory, oil well drilling, electroplating, mining, textile, pulp and paper industries, are among toxic heavy metal ions, which pose toxic effects to human, plants, microorganisms, and aquatic lives. This review work is aimed at biosorption of hexavalent chromium (Cr(VI)) through microbial biomass, mainly bacteria, fungi, and microalgae, factors influencing the biosorption of chromium by microorganisms and the mechanism involved in the remediation process and the functional groups participated in the uptake of toxic Cr(VI) from contaminated environments by biosorbents. The biosorption process is relatively more advantageous over conventional remediation technique as it is rapid, economical, requires minimal preparatory steps, efficient, needs no toxic chemicals, and allows regeneration of biosorbent at the end of the process. Also, the presence of multiple functional groups in microbial cell surfaces and more active binding sites allow easy uptake and binding of a greater number of toxic heavy metal ions from polluted samples. This could be useful in creating new insights into the development and advancement of future technologies for future research on the bioremediation of toxic heavy metals at the industrial scale.

1. Introduction

The leakage of serious heavy metals from human and industrial activities into wastewater has become a major environmental issue. Water pollution is a major threat to human, land, and aquatic animals and plants, and it is attracting the attention of many researchers and scientists. The main reason for this may be traced back to the increase in different industrial activities which takes great part in producing plenty of waste and untreated water throughout the world. It has been emphasized that the water pollution induced from the large amounts of industrial effluents without proper treatment before disposing to the water bodies is causing a serious threat to the entire human health [1], for example, water contaminated with atorvastatin (a drug used as cardiovascular ailments) has been causing adverse health effects such as myopathy, rhabdomyolysis, memory loss, forgetfulness and confusion, liver and muscle problems, diabetes, cognitive loss, neuropathy, pancreatic, and hepatic and sexual dysfunctions [2]. Various industrial, agricultural and domestic wastes dumped to water bodies can seriously pollute, and the potential contaminants can cause different diseases such as diarrheal diseases, vector borne diseases, cutaneous diseases, blindness, paralysis, and liver diseases (Chowdhary et al.). In general, organic dyes from textile and painting industries [1, 3], different drugs from pharmaceuticals [2], inorganic pollutants and polysaccharides from distillery industries [4], smoke and organic

wastes from pulp and paper industries [5], and wide varieties of heavy metals produced from metallurgical and electroplating, chemical, refractory brick, tanneries, wood preservation, pigments and dyes, textiles, etc., [6] are getting great environmental challenge and threatening the health of humans. Hence, great emphasis has been given by many researchers to heavy metals as they are highly toxic even at very lower concentration and continuously discharged to water bodies due to growing industrial activities.

Typically, heavy metals are those metallic elements and some metalloids with density higher than 5 gcm^{-3} [7]. Chromium (Cr), cobalt (Co), cupper (Cu), cadmium (Cd), arsenic (As), gallium (Ga), germanium (Ge), iron (Fe), mercury (Hg), lead (Pb), nickel (Ni), thallium (Tl), selenium (Se), and manganese (Mn) are some commonly known heavy metals [8]. They cover most transitional metals, basic metals, some metalloids, lanthanides, and actinides, having high molecular weight, atomic number, and specific gravity. Various industrial sectors such as electroplating, tanneries, pulp and paper, textile, metallurgies, mining sectors, and dying and painting industries extensively consume different heavy metals for diverse purposes [9–11]. These and other heavy metals are being continuously discharged from the manufacturing industry into living environments, especially water bodies at concentrations in excess of the limits set by regulatory agencies. Heavy metal exposure has been one of the big global concerns due to its high toxicity, high bioaccumulation in the human body and food chain, the essence of nonbiodegradability, and most likely human carcinogenicity [12].

Heavy metals are reported to pose toxic effect in humans (causing different cardiovascular, inflammatory, and respiratory diseases) and other animals [8, 13]; plants (disturb photosynthesis, production, and slow the growth) [14]; microorganisms (affect morphology, metabolism, and growth) [15]; and aquatic lives (affect reproduction and even death) [8]. Among toxic heavy metals, chromium is ranked among the top sixteen toxic contaminants that have adverse effects on human health as an essential metal [16]. Chromium is being used in various manufacturing sectors, such as the metallurgical, steel, tannery and cement industries, textiles, dying industries, which are major sources of pollution. Chromium can exist in various oxidation states among which, hexavalent Cr(VI) and trivalent Cr(III) states are most stable one and are most commonly discharged in to water bodies from industrial activities [17, 18]. The hexavalent form is especially one of the most significant sources of environmental contamination and is documented for its nephrotoxic malignant neoplastic disease and is well known for its toxic, carcinogenic, and mutagenic effects on humans and other living organisms [19, 20].

Various water treatment technologies have been reported for the removal of toxic substances from the water bodies. Some of which include biofiltration, membrane filtration, biological activated carbon, advanced oxidation process, ion-exchange resins, and iminodiacetic acid-carbon nanotubes [21–23]. To date, adsorption technologies such as covalent organic frameworks- (COFs-) based materials [24], metal organic framework (MOF) [25], porous geopolymers [26], biochar [27], chitosan-based adsorbents [28], 3D porous aerogels [29], agricultural and industrial wastes [30], nanomaterials [31], and biosorption [32] have been studied to remove toxic heavy metal contaminants from wastewater. More recently, efficient removal of Cr(VI) from the contaminated solutions has been reported. To mention, Zhu et al., [33] reported efficient removal of Cr(VI) using magnetite immobilized with *Lysinibacillus* sp. (a Gram-positive, mesophilic, rod-shaped bacterium commonly found in soil) and studied the mechanisms and performance to be applied in large scale. On other hand, magnetic chitosan modified with graphene oxide nanocomposite has been employed for the removal of Cr(VI) from waste water where the synthesized adsorbents exhibited varied removal efficiency at solution pH of 2 [34].

The simple, inexpensive, and ecofriendly solution that can be performed over a wide range of experimental conditions for the detoxification and elimination of Cr-pollutants is bioremediation, which uses indigenous microorganisms [32]. Most bioremediation technologies such as biosorption produce less or no secondary wastes during removing toxic heavy metal ions [35] as secondary wastes may require further technologies to be managed. The processes by which microorganisms deal with toxic metals are biosorption, bioaccumulation, and enzymatic oxidation/reduction [36]. Among bioremediation techniques, biosorption process using microbial biomass is reported to be simple, fast, economical, efficient, and also effective at industrial scales. Hence, this review work is focused on hexavalent chromium removal by biosorption using microorganisms, the mechanism involved in the removal process and the functional groups participating in the uptake of hexavalent chromium ions. In addition, an emphasis has also been given to some factors that affect haste, efficiency, and effectivities of biosorption process. In general, wide varieties of toxic heavy metals are continuously discharged into water bodies due to growing industrial and agricultural activities, and different emerging treatment technologies have been adopted to overcome such problems.

2. Overview of Heavy Metals

2.1. Industrial Heavy Metals as Major Water Pollutant. Most commonly, wastes can be discharged into the aquatic environment as domestic, agricultural, industrial, and radioactive wastes. Among these, industrial operation accounts for the largest portion of either emission of gases and discharge of different effluents. One of the main sources of water contamination is industrial wastewater, which contains high levels of heavy metals and other permanent hazardous substances. The rapid development of science and technology has taken the sector to a new stage of growth [37]. The key reasons for high pollution problems in the waste disposal field of the industry are waste toxicity and postdisposal behavior, inadequate treatment procedures, insufficient disposal, bad planning, and management of disposal sites. Wide varieties of heavy metals have been continuously discharged from industrial processes into water systems (both surface and underground waters) in

different ways (as shown in Figure 1). Most commonly, Cr, Hg, Cd, and Pb from tanneries [9]; Cu, Pb, Zn, Hg, etc., from mining [40], Hg, Pb, Cd, Zn, Cu, Cr, As, and Ni from coal production [11]; Cd, Cr, Cu, Fe, Mn, Ni, etc., from textile manufacturing [39]; Fe, Zn, Cu, Cr, Cd, Mn, Ni, and Pb from pulp and paper [38], and others such as metal refining, electroplating, petroleum production, fertilizer, pesticide, and painting industries [10] also generate heavy metals which are discharged into environment with concentration higher than the proclaimed level by regulatory organizations.

Amidst various manufacturing industries, the tanning industry is an important contributor to the economy and, in particular, provides large-scale job opportunities to unskilled and/or trained individuals. Despite the great role of the leather industries in the social and economic development of the world, it is also the leading contributor of water contamination by heavy metals, mainly chromium. Wastewater from tanneries is primarily characterized by high levels of chemical demand for oxygen (COD), biochemical oxygen demand (BOD), turbidity, suspended solids (SS), sulfides, and chromium [41]. Huge quantities of heavy metals have been released worldwide into rivers or freshwater due to rapid global population growth, the expansion of global industrial and domestic activities, and agricultural development [42]. Heavy metals released into water bodies by waste have an incorrigible effect on the aquatic environment and damage the ability of an aquatic body to self-purify [43, 44].

Among several heavy metals generated from different industrial activities, the toxicity and health effects of chromium (Cr), which is the dominant heavy metal from tanneries, attracted the attention of researchers. Chrome tanning is one of the leather tanning industry's most commonly used methods since it provides a product with the highest value at a fair price. As a result, this process accounts for about 8% of the weight of the leather as chromium salt is applied [45]. Although the tanning process is highly important in improving the quality and acceptability of leather products, it comprises several chemical compounds such as acids, vegetable tannin, mineral salts, such as chromium sulfate, and fish or animal oil, some of which are reported to be potentially toxic to human, plants, and aquatic lives [43, 46–48]. Generally, industrial activity takes higher part in producing toxic heavy metals as waste, which directly or indirectly enter into water bodies, and Cr(VI) highly produced from tanneries is getting great challenges to be easily removed from water bodies.

2.2. Definition, Sources, Speciation, and Properties of Chromium

2.2.1. Definition and Properties of Chromium. Chromium is a steely-grey, lustrous, hard and brittle transitional metal symbolized Cr, having atomic number 24, molecular weight 51.9961gmol^{-1} and density 7.19 g/cm^3 at 20, melting and boiling points at 1,907 and 2,672, respectively. In the modern periodic table, Cr is the first element of group 6 and corresponds to period 4, a d-block element with an electronic configuration of [Ar]3d^{5}4s^1 and is located between vanadium and manganese. Naturally occurring chromium is composed of three stable isotopes: ^{52}Cr, ^{53}Cr, and ^{54}Cr, with ^{52}Cr being the most abundant (83.789% natural abundance) and is the 21st commonly abundant element in Earth's crust at around a hundred ppm [49, 50]. Cr(III) and Cr(VI) are the most commonly occurring oxidation states of chromium and are reported to be more toxic to animals, plants, and humans among its several oxidation states [50–54].

In a very simple and oxidizing medium, the trivalent chromium cation occurs only in a strongly acidic and decreasing medium, while hexavalent chromium occurs as CrO_4^{2-} anion [55]. Chromium metal with various oxidation states exhibit different characteristics, including a metallic luster, brittle and tough, steely-grey color, tarnish resistance, and high melting and boiling points (1907 and 2671, respectively) [56]. Also, chromium belongs to the refractory metal group, which includes all metals with a melting point higher than platinum (1,772°C). As a whole, chromium, the most known transition metal located in the 6th group and 4th period of the periodic table is abundant in the Earth crust and occurs as different stable isotopes and the various oxidation states of the metal determine its properties and characteristics.

2.2.2. Sources of Chromium. Chromium and associated chemical compounds can mainly originate naturally or anthropogenically.

(1) Natural Sources. From the two most stable oxidation state of chromium (i.e., Cr(III) and Cr(VI)), the trivalent one exists naturally in a complex form with the chromite ore (FeCr2O4), a mineral which exists in mafic and ultramafic rocks and is a complex of varying proportions of magnesium, iron, aluminum, and chromium [57–60]. The naturally released Cr(III) ion can most probably oxidize into a highly toxic form (i.e., Cr(VI)) through microbial intervention and geochemical processes [57, 60]. Also, geologic parent materials or rock outcroppings and volcanoes are the most significant natural source of chromium pollution in the environment [61]. Chromium in rocks, mineral soils, and freshwater is usually a dispersed geochemical component. Cr is also present in all sorts of environmental elements, including air, water, and soil, of course, but in limited amounts [46, 62, 63].

(2) Anthropogenic Sources. Besides the existing natural process, various industrial activities produce chromium into the water bodies which have been a great threat for aquatic and terrestrial life and continued to be a concern of many researchers worldwide. Accordingly, many industrial processes employ chromium and its compounds for varied purposes. Among those manufacturing industries metallurgical, refractory, oil well drilling, metal plating, mining, textile, pulp, petroleum, chemical production, and leather consume chromium in varied amounts for different applications [64–69]. Among the most commonly existing oxidation states, Cr(VI) concentrations have increased dramatically in both marine and terrestrial ecosystems over

Figure 1: Different industrial activities discharging various heavy metals to the water systems [9, 11, 38, 39].

the past few centuries. It originates mainly from dyeing, textiles, steel fertilizers, photography, electroplating, manufacturing, packaging, and leather tanning and finishing [70].

In general, the most stable species of chromium, Cr(III) and Cr(VI), are produced both naturally and anthropogenically. The former one is mainly produced naturally by rock weathering, volcanic activities, and other geologic process, which gets oxidized to highly toxic Cr(VI) form by microorganisms in the environment. The hexavalent form highly produced through anthropogenic process by human activities rather than natural process.

2.2.3. Oxidation States of Chromium. Chromium may exist between 0 and VI in a variety of chemical varieties; however, trivalent and hexavalent chromium alone is sufficiently stable to occur in the environment [71, 72]. Chromium occurs in various chemical forms in soil (primarily as chromite (Cr(III)) and chromate (Cr(VI)), which vary greatly in terms of their biogeochemical actions [50]. Chromium species are commonly present in environmental water in two separate oxidation states, especially Cr(VI) and Cr(VI), with contrasting physiological effects. Cr(III) is considered to be an important trace element for the maintenance of the efficient metabolism of glucose, lipids, and proteins in mammals. Chromium at 0 oxidation state does not exist naturally in the Earth's crust and biologically inert, while Cr(II) can be easily oxidized into Cr(III) in the presence of air and is chemically unstable [73, 74]. This implies that different oxidation states of chromium have possessed varied properties and toxicity. On the other hand, Cr(VI) may be toxic and cancerogenic to human biological systems. Therefore, speciation of Cr(VI) and Cr(III) is important to determine toxicological actions [75, 76]. In general, though chromium exists in various oxidation states (0 to VI), only the trivalent and hexavalent forms are relatively more stable, and the difference in the oxidation state led to variation in properties and toxicity of the metal.

2.2.4. Importance of Chromium. Despite their toxicity, carcinogenicity, and environmental threats at elevated concentrations, chromium and its compounds at a limited amount are applied for various purposes in different units. For instance, Cr(VI) and its compounds have been employed as pigments for photography and pyrotechnics, electroplating, tanning, textile production, coloring, painting, inks, wood preservation, and plastics [77, 78]. Besides, chromium and its preparations are widely used in many industrial processes such as chrome plating, wood preservation, textile dyeing, pigmentation, chemical production, paper, and tanning [79–81]. As laboratory reagents and as production intermediates, chromium compounds, such as hexavalent chromium, are documented. The metallurgical, chemical, and refractory brick industries are major industries that use chromium. Chromium is distinguished by its high resistance to corrosion and its hardness. Thus, the

invention that steel could well be made to be extremely resistant to corrosion and discoloration by adding Cr to render stainless steel was a serious advancement. Besides Cr electroplating, this application is the highest volume user of the metal. The ore produces Cr and ferrochromium. Cr(VI) salts are used for timber preservation because of their toxicity [71]. In general, Cr in the industry is commonly used in plating, alloying, animal hide tanning, water corrosion inhibition, textile dyes and mordants, pigments, ceramic glazes, refractory bricks, and pressure-treated lumber [82].

Besides various industrial and laboratory applications, chromium, particularly trivalent chromium is nutritionally useful at the trace level. For example, a wide range of nutrients, such as coffee, nuts, green beans, egg yolks, whole-grain products, high-bran breakfast cereals, broccoli, meat, Brewer's yeast, and some brands of wine and beer are enriched with trivalent chromium [81, 83]. The recommended daily consumption of chromium according to the United State National Academy of Sciences is 50–200 μg for adult men and women [81], but this range was recently lowered to 35 μg and 25 μg for adult men and women, respectively, according to Food and Nutrition Board at the Institute of Medicine [84]. However, more recently, the European Union of Food and Safety Administration had put weekly acceptable intake only, which is 300 μg/kg of body weight per week [85]. It has been documented that chromium is important in protein, carbohydrate, and lipid metabolism, and its deficiency leads to glucose intolerance and insulin resistance [84, 86]. With this regard, daily intake of chromium at a reduced concentration (trace level) is associated with different medical applications. For instance, daily consumption of low to moderate amount of chromium helps fight against type II diabetes and obesity [83, 84, 86], and chromium-containing alloy has also dental applications such as restoration, orthodontic appliances, and implants [83]. As a whole, chromium and its compounds are extensively used in different industrial activities and as reagents in laboratories, and in trace amount, the trivalent form is important candidates in nutrition.

2.2.5. Chromium Exposure and Toxicity. Chromium and its compounds, particularly chromates, enter into the human body through different pathways. Accordingly, inhalation, ingestion, and dermal contacts are the major integral exposure routes for chromium, and the exposure may be acute (14 days), intermediate (75–364 days), or chronic (365 days) [47, 87]. Moreover, anthropogenically released chromium causes toxic effects in most exposed populations by inhalation of polluted air or drinking of chromium contaminated water [88, 89]. Owing to its characteristic toxicity and carcinogenicity, the United State Environmental Protection Agency (US-EPA) and US Occupational Safety and Health Administration (OSHA) have put the total chromium exposure limits for drinking water standards which are 100 μg/L and Cr(VI)-timed weighted average for a normal workday exposure which is 5 μg/L [89]. Furthermore, the concentration of 0.510^{-4} μg/L is the maximum permissible limit for the discharge of total chromium into the surface and portable water according to US-EPA and European Union [90].

The pattern of toxicity and accumulation of heavy metals in the atmosphere is a significant danger to the health of living organisms [15]. Heavy metal exposure has been one of the big global concerns due to its high toxicity, high bioaccumulation in the human body and food chain, the essence of nonbiodegradability, and most likely human carcinogenicity [12]. Among various heavy metals ions, the most stable chromium species (Cr(III) and Cr(VI)) are known to be toxic to aquatic and terrestrial life of which the hexavalent form is reportedly highly toxic and carcinogenic. The toxic, mutagenic, and other biological effects of chromium depend on its oxidation states. Accordingly, hexavalent chromium Cr(VI) is more harmful, cancerous, teratogenic, mutagenic, and movable than trivalent chromium Cr(III) because of its oxidizing existence [91, 92]. Even though the trivalent form is less toxic relatively, it induces its harmfulness by oxidizing into the most toxic hexavalent form in the presence of oxygen in the environment [65]. According to [47], the exposure to dichromate and chromates, which are oxyanion of chromium in +6 oxidation states and trivalent chromium released from alloys and Cr-plated objects, can cause dermatitis in humans. In synthetic pigment and industrial wastewater, Galvanometry and electricity, film and photography, plating and electroplating, metal washing, mining, and leather, chromium is the primary contaminant [93].

Chromium, especially, the hexavalent species poses its toxic effect through different mechanisms of actions. Among those, reducing activity or efficiency of the immune system; competing at enzyme activity cofactor fixation sites; inhibiting important enzymes such as oxidative phosphorylation and altering cell structures, mainly in the lipoprotein area of the membrane, are the key ones. Hexavalent chromium demonstrates high toxicity through its DNA-polymerase enzyme interface due to nasal irritation and ulceration, hypersensitivity reactions and contact dermatitis, acute bronchitis and emphysema, liver and kidney injury, internal hemorrhage, lung and skin cancer, and DNA damage [90]. Cr(VI) more readily enters the cell, but transfers multiple processes in the blood and gets reduced into Cr(III) in the human body [47]. Cr(VI) is removed from the body, while the chromate ion is transferred to the cell through a transport mechanism by which sulfate and phosphate ions also join the cell [52]. The presence of these ions in a cell can trigger oxidative stress, which is highly responsible for several chronic, neurodegenerative, cardiovascular, and other age-related diseases. In general, the augmented levels of oxidative stress, DNA adduct development, and chromosome breakups are some of the major mechanisms by which Cr(VI) cause cellular damage [89]. In due course, humans are exposed to chromium through ingestion, inhalation, and derma contact, and the exposure may be acute, intermediate, or chronic in general.

2.2.6. Toxic Effects of Chromium in Humans. Based on the epidemiological studies linking Cr(VI) to lung cancer, the International Agency for Research on Cancer (IARC) of the World Health Organization (WHO) classified Cr(VI)

compounds as group one human carcinogens with multiple complex mechanisms of actions [89, 94, 95]. Human exposure to Cr(VI) can cause allergies, irritations, eczema, ulceration, nasal and skin irritations, eardrum perforation, respiratory tract disorders, and lung cancer [96]. Cr(VI) exposure may lead to cytotoxicity, genotoxicity, and carcinogenicity of Cr(VI)-containing compounds, DNA mutations, and chromosomal damage at different stages, as well as to oxidative protein changes [47, 81, 97, 98]. In addition, inhaling a high level of hexavalent chromium can cause nose ulcer, irritation to the lining of the nose, anemia, irritation and ulcer in the small intestine and stomach, as well as other respiratory complications such as wheezing, coughing, nasal blockage, and facial erythema [13, 87, 99]. Eventually, repeated and high exposure of chromium and associated compounds, especially those containing hexavalent ions can severely cause several hazards, of which most are categorized under those stated in Figure 2.

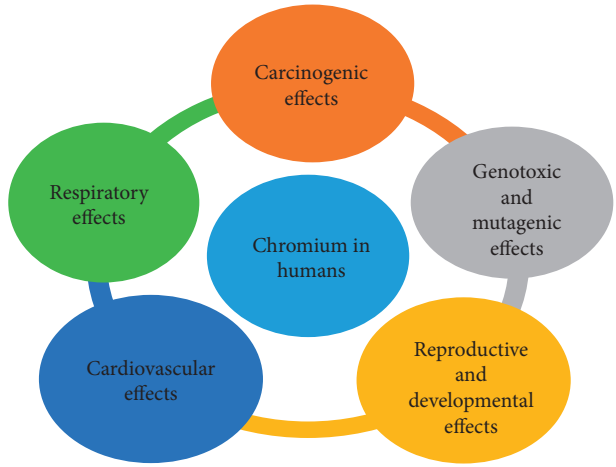

Figure 2: Toxicological effects of chromium on humans [61].

2.2.7. Toxic Effects of Chromium in Microorganisms. Biologically, chromium ions are important nutrients as they involve in electron transport reaction in biological systems; however, microorganisms are also highly sensitive to both deficiency and excessiveness of chromium and its compounds just like plants and humans [61]. Chromium toxicity affects microbial population size, diversity, and behavior, as well as their genetic structure. Microorganism exposure to hexavalent chromium can cause alteration in morphology of both Gram-positive and Gram-negative bacteria. It disturbs the morphology, metabolism, and growth of microorganisms by altering the structure of nucleic acid, disrupting the cell membranes, causing functional damage, inhibiting enzyme activity and oxidative phosphorylation, and causing lipid peroxidation and osmotic balance [15]. Chromium causes bacterial cell elongation and cell enlargement and prevents cell division, which is essentially responsible for cell growth inhibition [61]. In general, both shortage and exorbitance of chromium can cause adverse effects in microorganisms such as disruption of the growth and cell functions are retarded.

2.2.8. Toxic Effects of Chromium in Plants. Chromium compounds released naturally and/through different anthropogenic activities have been continuously discharged into the soil, in which different flora grow. Chromium compounds most commonly exist in the soil in the form of $HCrO_4^-$ and CrO_4^{2-} which get easily absorbed by the plants and rapidly trickled down into the deep layers of soil, thereby endorsing surface and groundwater pollution [85]. Among various oxidation states, Cr(III) is considered to be less harmful, preventing its leaching into groundwater or its absorption by plants due to its extremely low solubility, while Cr(VI) has been shown to cause substantial harm to living cells [100]. Although some crops are not affected by low levels of Cr ($3.810^{-4}\,\mu$M) [101], chromium compounds are highly toxic to most plants and are harmful to their production and growth. But Cr(VI) can be extremely poisonous to plants in concentrations as little as 5 mg/kg in soils and 0.5 mg/L in solution according to Elahi et al. [85]. A reduction in nutrient uptake and photosynthesis is also correlated with Cr(VI), which contributes to slow plant growth. Several physiological, morphological, and biochemical processes are seriously disrupted and cause the production of reactive oxygen species in plant cells. Cr toxicity is indicated in the form of chlorosis and plant necrosis [62]. The major toxic effects of chromium in plants are summarized in Figure 3.

Chromium also affects the growth of leaves, the main photosynthetic plant organ. Increasing chromium concentration leads to a significant reduction in the leaf area and leaf biomass, which is accompanied by decreased photosynthesis and induction of chlorosis and necrosis of leaves. Under Cr exposure, many destructive processes take place in leaves. These include suppression of chlorophyll synthesis, disruption of chloroplast ultrastructure, inhibition of photosynthetic electron transport, and release of magnesium ions from the molecule of chlorophyll [102]. Symptoms of Cr(VI) poisoning in plants include reduced plant growth, leaf deformation and necrosis, 88 chlorosis, decreased enzyme activity and nutrient absorption and transport, damage to root tissue, decreased photosynthesis, lipid peroxidation, DNA strand break, and chromosome aberration [81, 103]. Thus, chromium can inhibit photosynthesis, seed germination, and nutrient uptake and affect the growth and functionality of its parts in general.

3. Chromium Removal Methods

Several methods of removing heavy metals from polluted water have been used [104–107]. Heavy metals such as chromium have been removed from polluted environments such as wastewater via several conventional physicochemical procedures such as chemical precipitation, electrolysis, ion exchange, floatation, coagulation and flocculation, membrane filtration, reverse osmosis, adsorption, and photocatalysis [56, 108–114]. The removal of chromium by such traditional methods are inefficient during the reduction of heavy metals at very low concentrations of 10–100 mg/L so that the metal ions are not completely removed and also the

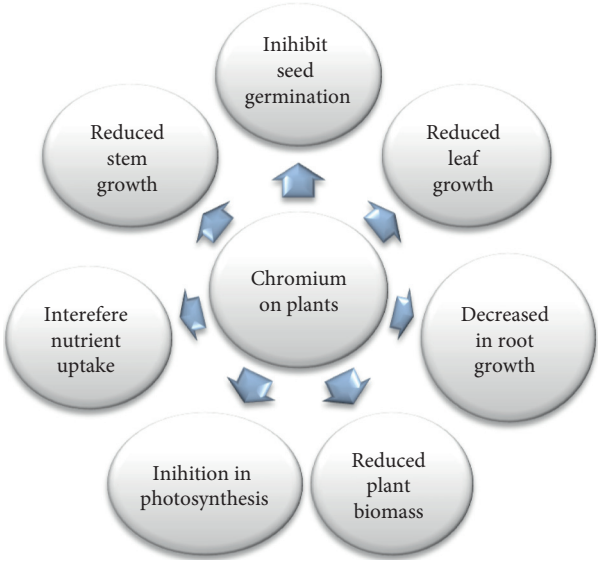

Figure 3: Toxic effects of chromium in plants [50].

methods are very costly [109, 115, 116]. In addition, these conventional methods generate large quantities of secondary waste (sludges) and other toxic products that may require other technologies to be brought to nontoxic, biodegradable, and environmentally benign forms.

Consequently, the focus has been shifted to biological remediation approaches having relatively high removal efficiency, less cost and environmentally benign. Biotransformation and biosorption are widely used technologies that utilize the ability for heavy metal transformation or adsorption by microorganisms or plants [56, 117]. Technologies for the biological removal of heavy metals use biomass to remove chromium from industrial waste and are cost-effective, environmentally safe and easy to operate [56, 118]. The alternative and environmentally sustainable form of chromium biosorption is the biological one. Potential biological methods (i.e., biosorption, bioaccumulation, oxidation/reduction, leaching, precipitation, volatilization, decay, and phytoremediation) have been employed to remove and detoxify toxic heavy metals from contaminated water and sediments. In addition, the options for the recovery of metals sequestered by biosorbents (use of suitable desorbing agents) and microbial biomass are identified [119]. Though different chemical and physical methods have been applied as removal technique, the biological remedy using microorganisms attracted the attention due to economic feasibility, simplicity, efficiency, and production of less or no secondary wastes in general.

3.1. Biological Method of Chromium Removal. The biological method of heavy metal removal involves the decomposition and/reduction of toxic heavy metal ions to relatively less and/nontoxic forms by using the living and/or dead biomass of microorganisms (such as bacteria, fungi, algae, and yeast), industrial or agricultural wastes, naturally occurring materials (such as clay, chitin, chitosan, lignin, and zeolite), and different parts of plants. A potentially useful method for the detoxification of Cr contamination and bioremediation of polluted waste is the biological reduction of Cr(VI) to Cr(III) [100]. Chromium removal by microbial species is a sustainable development solution that is healthy and environmentally friendly. These techniques are conceivably employed by using different microbial biomass mainly bacteria, fungi, and algae. The biosorption of Cr(VI) using fungal, algal, or bacterial biomass (growing, resting, and dead cells) and biological and agricultural waste materials has been described as a possible alternative to the current conventional methods of industrial wastewater detoxification [20]. Chromium-resistant microorganisms are responsible for/to the biological reduction of Cr(VI) to less mobile Cr(III), and their consequent precipitation may be an important method of detoxification of polluted Cr(VI) sites and have a possible application for bioremediation [96]. Microorganisms remove heavy metals in such a way that they utilize metal ions for their advancement and by converting them into carbon dioxide, methane, water, and biomass through enzyme-catalyzed metabolism of poisonous substances [120].

In the microbial remediation process, microorganisms (such as bacteria, fungi, algae, and yeasts) are stimulated to readily degrade the environmentally toxic pollutants to ecofriendly or safe levels in soil, subsurface materials, water, sludge, and residues [35]. It has been reported that different microbial remediation techniques such as biosorption, bioaccumulation, biotransformation, and bioleaching have been employed to remove chromium and other heavy metals from industrial wastewaters [92]. The bioremediation process recently reported as the dominant friendly mechanism for heavy metal removal from a polluted environment is summarized in Figure 4. Bioaccumulation, also called active biosorption, is a metabolism-dependent mechanism in which hexavalent chromium (Cr(VI)) is transported across the membrane into the cell by cellular energy in living biomass only [92]. The bioaccumulation process occurring in living microorganisms involves the following steps [56, 121]. Initially, potentially toxic heavy metal ions get attached to the surface ligand of the cell. Then, the metal-ligand complex formed at the surface of the cell is transported inside the cell by transporter protein. Finally, transported complexes intracellularly interact with metal-binding proteins (such as metallothionein and phytochelatins), where precipitation, methylation, and other process takes place. However, the process is limited to the living cells only and inhibits microbial cell growth at a relatively higher metal concentration [92]. Finally, the living and dead microbial biomass, mainly that of bacteria, fungi, and algae ecofriendly degrade and remove toxic chromium ions by the process of biosorption, biotransformation, and bioaccumulation.

3.1.1. Reduction of Cr(VI) to Cr(III). As it has been discussed in preceding sections, hexavalent chromium is more toxic to plants, animals, and aquatic species when compared to the trivalent species. Hence, transforming the more toxic Cr(VI) species from the solution to the less toxic one can take place

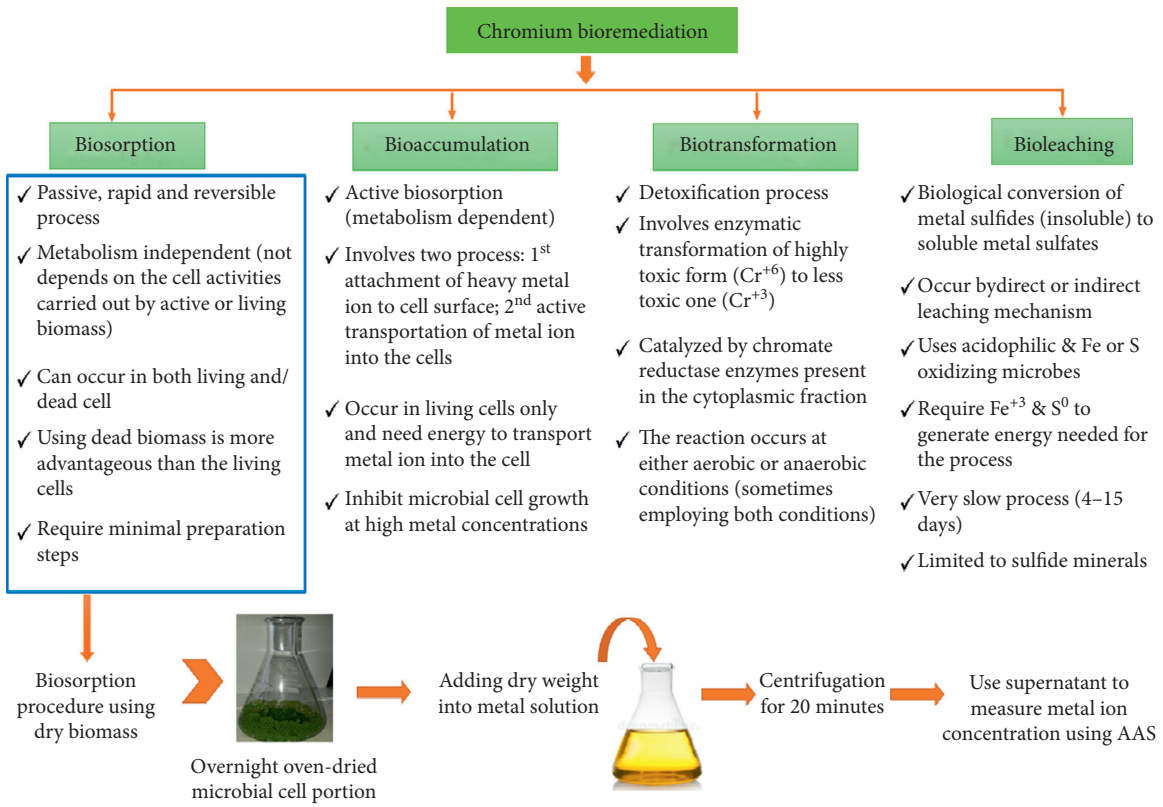

Figure 4: Heavy metal bioremediation processes by microorganisms [87, 92, 121–123].

by different process through varied conditions. In addition to extracellular adsorption and intracellular accumulation, Cr(VI) can importantly transform and reduce to less toxic Cr(III) species by chromium reductase enzyme [63, 124]. More importantly, the biomass surface with electron donating agents such as hydroxyl groups, amine groups, and secondary alcohol groups interact with Cr(VI) favorably at acidic pH and is reduced to Cr(III), which subsequently binds to the negatively charged functional groups (such as sulfonate group and carboxyl group) [7]. Even though the reduction of hexavalent chromium to the trivalent one most of the time takes place in living biomass, it has also been observed in nonliving microalgae biomass, which might due to the release of glutathione [63]. In this regard, Cr(VI) reduction at acidic pH condition using dead microalgae biomass is as follows [125].

Therefore, in addition to biosorption and bioaccumulation, hexavalent chromium can also be removed from aqueous solution by being enzymatically reduced and transformed in to less toxic species (such as Cr(III)) by living or dead microbial biomass at different favorable conditions.

3.1.2. Hexavalent Chromium (Cr(VI)) Biosorption. In contrast to the bioaccumulation process, biosorption is a metabolism-independent mechanism, which can occur in both living cells (but not depend on cell activities) and dead microbial biomass [92, 120, 121, 126]. In biosorption mechanisms, the toxic heavy metal ions such as Cr(VI) extracellularly bind to various functional groups of the microbial cell wall (as shown in Figure 5), which are removed either by surface precipitation, ion exchange, or complexation/chelation process [92, 121, 127]. The compositions and structures of the cell wall vary across different microorganisms. For instance, the bacterial cell wall composed mainly of peptidoglycan [128], but that of fungi is glucans, chitin, glycoproteins, melanin [129], and the algae cell wall is composed mainly of alginate, mannans, and sulfonated polysaccharides [130].

Hence, the extensive mechanism for the biosorption process is very complex since it depends on the types of biomasses going to be used, the functional groups of the microbial cell wall, its structure, and extracellular polymer substances secreted by microorganisms [131–133]. The biosorption process is reported to have various advantages over other bioremediation mechanisms for toxic heavy metal removal from polluted environments. The presence of multifunctional groups and uniform distribution of binding sites on the cell surface, no need for additional nutrients (chemicals), minimal preparatory steps, low cost, high efficiency, regeneration of biosorbent, and possibility of metal recovery are some advantages of the biosorption process [90, 121]. Owing to these and other related advantages, the focus has been given to the biosorption process using different microbial biomass such as bacteria, fungi, and microalgae for the removal of heavy metals particularly hexavalent chromium. Different microbial

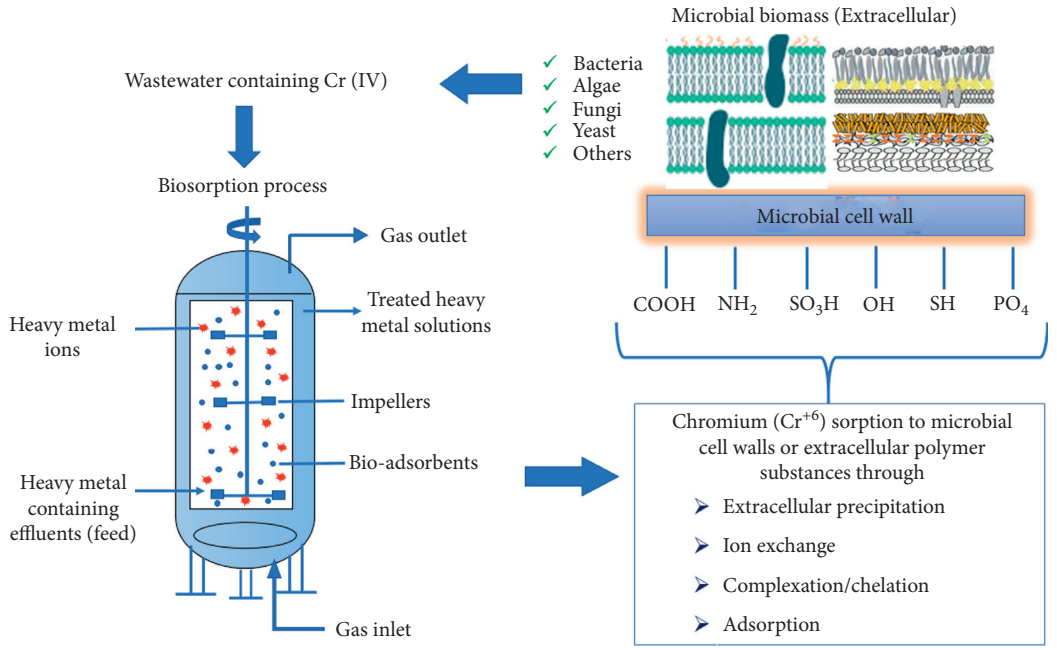

Figure 5: Hexavalent chromium (Cr(VI)) biosorption process by microorganisms [90].

Table 1: Various microorganisms used for biosorption of chromium.

S.No.	Species	IC (mg/L)	Dose (g/L)	pH	Time (hr)	% Removal	References
	Bacteria						
1	*Bacillus salmalaya*	—	—	3	0.83	20.35 mg/g	[134]
2	*Bacillus* sp.	—	—	—	72	75	[135]
3	*Bacillus* spp.	1	0.25	2	5	99	[110]
4	*Enterobacter cloacae*	0.001	—	—	120	81	[19]
5	*Chelatococcus daeguensis*	15	—	7	24	94.42	[136]
6	*Micrococcus* sp.	100	—	7	24	90	[137]
7	*Planococcus* sp.VITP21	142.8	—	6.8	12	83	[138]
8	*Pseudomonas alcaliphila* NEWG-2	200	—	7	48	96.60	[139]
9	*Halomonas* sp. DK4	100	—	6	48	81	[140]
10	*Klebsiella* sp	100	—	9	72	63.08	[141]
11	*Sinorhizobium* sp. SAR1	—	7.5	1	—	285.71 mg/g	[142]
	Fungi						
1	Consortium *of Penicillium commune, Paecilomyces lilacinus, Cladosporium perangustum, Cladosporium perangustum*, and *Fusarium equiseti*	—	—	4	24	100	[143]
2	*Fusarium* sp	—	—	—	—	100	[144]
3	*Aspergillus Niger*	18.125	4	3	168	96.3	[145]
4	*Aspergillus flavus*	18.125	4	3	168	92.8	
5	*Aspergillus fumigatus*	18.125	4	3	168	90.1	
6	*Aspergillus nidulans*	18.125	4	3	168	86.7	
7	*Aspergillus heteromorphus*	18.125	4	3	168	83.7	
8	*Aspergillus foetidus*	18.125	4	3	168	78.6	
9	*Aspergillus viridinutans*	18.125	4	3	168	74.4	
10	Consortium of *Aspergillus lentulus, Aspergillus terreus*, and *Rhizopus oryzae*	100	—	6.5	96	100	[146]
11	*Aspergillus lentulus*	100	—	6.5	120	83.11	
12	*Aspergillus terreus*	100	—	6.5	96	95.57	
13	*Rhizopus oryzae*	100	—	6.5	168	25.34	
14	*Aspergillus versicolor*	50	—	6	—	99.89	[147]
15	*Rhizopus oryzae*	400	—	7	72	91.15	[148]

Table 1: Continued.

S.No.	Species	IC (mg/L)	Dose (g/L)	pH	Time (hr)	% Removal	References
		Bacteria					
16	*Aspergillus sp*	100	—	5	24	92	[137]
17	*Cladosporium cladosporioides*	504	0.5	1	288	491.85 mg/g	[149]
18	*Trichoderma* sp.	200	0.0016	5	2	96.38	[150]
19	*Aspergillus terreus*	1000	10	1	24	54	[151]
20	*Pleurotus ostreatus*	50	—	—	0.367	100	[152]
21	*Pleurotus ostreatus*	—	0.2	5	2	20.71	[153]
		Microalgae					
1	*Scenedesmus* sp.	10	10%(w/v)	1	1.5	92.89	[154]
2	*Pseudanabaena mucicola*	—	—	2	—	71	[155]
3	*Pseudopediastrum boryanum*	10	2	2	—	70	[112]
4	*Scenedesmus sp.*	—	—	—	—	98	[156]
5	*Chlorella colonials*	6.07	2.7		108	97.8	[157]
6	*Chlorella sorokiniana*	100	0.2	6.2	72	99.6793	[158]
7	*Chlorella sp.*	—	—	—	96	34	[159]
8	*Chlorella vulagris*	100	1.2	3	1	99.75	[160]
9	*Scenedesmus* sp	10	10%(w/v)	1	2	93.1	[154]
10	*Spirulina platensis*	150	0.2	1	1.5	45.5 mg/g	[161]
11	*Isochrysis galbana,*	—	10 mL	5	2	335.27mg/g	[162]
12	*Chlamydomonas* sp	250	1.5	4	0.5	91	[163]

biomass that has been employed for biosorption of chromium is summarized in Table 1.

The ability of microorganisms, for example, bacteria, fungi, and algae to remove heavy metal ions and, or to promote their transformation to less-toxic forms, has attracted the attention of various environmental scientists, engineers, and biotechnologists for many decades [10]. This is mainly because the process is simple, rapid, economical, and environmentally benign when compared to the physical or chemical techniques. In general, biosorption is metabolism-independent process, in which both living and dead cells can be used and the Cr(VI) ion extracellularly bind to various functional groups of the microbial cell wall, which are removed either by surface precipitation, ion exchange, or complexation/chelation process as typical mechanism.

3.2. Bacteria. Chromium removal by using bacterial strain is characterized as relatively rapid, economic, requires less energy, and the process requires less or no chemicals [62]. Bacterial species isolated from metal-contaminated environments are highly resistant to toxic heavy metals than those isolated from none-contaminated environments. Both Gram-positive and Gram-negative bacterial strain isolated from soil, water, and another chromate contaminated environment especially, effluents from tanneries and electroplating industries, has been employed for chromium biosorption [90, 164]. However, at a relatively high concentration, Gram-positive bacteria showed significantly good tolerance to toxic Cr(VI) when compared to Gram-negative bacteria [164]. Different bacterial species recently employed for chromium biosorption are summarized in Table 1. Due to their high surface-to-volume ratios and a high content of potentially active chemosorption sites such as teichoic acid in their cell walls, bacteria make outstanding biosorbent. Due to their small scale, their ubiquity, their capacity to grow under regulated conditions and their resistance to a broad variety of environmental circumstances, bacteria have been used as biosorbent. [165].

3.3. Fungi. Fungi are microbes that, due to the production of high biomass yields, are used as biosorbent for the removal of heavy metals. They are among the abundantly used microorganisms for biosorption of Cr(VI) since they are versatile, capable to adopt harsh environments, and can tolerate high hexavalent chromium concentration (above 10,000 mg/L) [90]. Fungal species are usually immune to higher metal ion concentrations [20, 165]. In fungi, Cr(VI) is adsorbed on the cell surface by forming a chemical bond with functional groups which are present on cell surface proteins [62]. The binding of hexavalent chromium to the fungal cell is owed to the chemical components, mainly proteins, lipids, and polysaccharides such as galactosamine, chitin and glycan, and the varieties of functional groups such as carboxyl (–COOH), phosphate ($PO4^{3-}$), amine ($–NH_2$), thiol (–SH), and hydroxide (–OH) groups [166]. Cr-fungi interactions have been extensively studied, mainly related to chromate tolerance in filamentous fungi and yeasts, and chromate reduction by yeasts. Fungi are usually less susceptible to metals than bacteria [66].

3.4. Microalgae. Algae are efficient and cheap biosorbents due to little nutrient requirement and their high productivities (i.e., high growth rate compared to the terrestrial

plants and can complete an entire growing cycle in every few days) [167]. Based on statistical analysis on algae potentiality in biosorption, it has been reported that algae absorb about 15.3%–84.6% which is higher as compared to other microbial biosorbents [168]. Heavy metal uptake by microalgae encompasses passive biosorption by dead biomass and active biosorption by living microalgae cells. In the process of passive biosorption, metal ions in the cationic form are physically adsorbed over the microalgal cell surface that contains functional groups such as hydroxyl (-OH), carboxyl (-COOH), amino (-NH_2), and sulfhydryl (-SH). During active biosorption, the metal ions are translocated across the cell membrane into the cytoplasm [169]. The binding of heavy metal ions to microalgae biomass is owed to the presence of multiple functional groups such as carboxylic acid, ether, amide, hydroxyl, and other carbonyls [170]. Among various binding functional groups, deprotonated sulfate and carboxyl groups as well as the monomeric alcohol and laminarin, are most likely responsible for the biosorption process [171]. Depending on its speciation, there are reports of tolerance or resistance of a small number of algae to Cr. The reduction of Cr(VI) to Cr(III) and reduced Cr uptake by algal cells is not likely to be involved in Cr(III) algal resistance [66].

In winding up, dead and living biomass of bacterial, fungal, and algal species are the most important, extensively used, relatively rapid, and economic process for removing toxic and hazardous metal ions like hexavalent chromium by biosorption.

4. Factors Influencing Chromium Biosorption by Microorganisms

4.1. The Influence of pH. It is a very important parameter. It affects the solubility of metal ions and binding sites of biomass [172]. Both biosorption and Cr(VI) reduction is dependent on the initial pH of the media. The carboxylic, phosphate, and amino groups are deprotonated at pH values greater than pKa, and therefore, negatively charged surface functional groups are able to bind positively charged metal ions, but metal ions no longer attracted to the biomass as the carboxylic, phosphate, and amino groups become protonated at pH values less than pKa [173]. Since Cr(VI) reduction is a proton consumption process, there is an increment in pH with Cr(VI) reduction. Hence, Cr(VI) reduction is generally higher at low pH values [88]. Indeed, the biosorption of chromium is highly favored as chromium anions are electrostatically get attracted to the carboxyl and amino groups on the surface of biomass, which are protonated at lower pH [110]. According to Ozer et al. [174] *Pediastrum boryanum* biosorbents showed its maximum adsorption of Cr(VI) at pH 2.0, but when the pH value raised, Cr(VI) removal efficiency was declined. This indicates that the pH of the reaction environment highly affects the heavy metal biosorption capacities of microbial biomass. The optimal conditions employed by different researchers to obtain good biosorption efficiencies by different microorganisms, mainly bacteria, fungi, and yeast biomass are given in Table 1.

4.2. The Influence of Temperature. The temperature of the adsorption medium could be important for energy-dependent mechanisms in metal biosorption by microbial cells [175]. The temperature is an important parameter for bacterial growth, which affects the enzymatic reactions necessary for Cr(VI) reduction [176]. Growth and reduction of Cr(VI) is negatively affected by extreme temperature which arises from the reduction in viability or due to the arrest of the physiological activity of the cell. Higher temperature results in protein denaturation and DNA damage as well as change in the structure of the membrane [177]. According to [178], with an increase in the temperature range of 30 to 40, the removal rate of Cr(VI) by strain *Desulfovibrio* CR-1 gradually increased to the same level, and the removal effect was best.

4.3. The Influence of Contact Time. Contact time is one of the most significant parameters for the effective use of the biosorbent for a practical application, and it has direct impact on the heavy metal removal efficiency. Metal ion removal efficiencies increase with increasing contact time and more or less remain constant after equilibrium achieved [179, 180]. Biosorption occurs rapidly if equilibrium is optimally attained within a few hours [181]. The metal adsorption of chromium (VI) by *Pantoea* sp. TEM18 increased rapidly during the first 5 min and remained nearly constant after 15 min. After this equilibrium period, the number of adsorbed metals ions did not significantly change with time [182].

4.4. The Influence of Initial Metal Ion Concentration. The initial concentration provides an important driving force to overcome all mass transfer resistance of metal between the aqueous and solid phases [172]. At higher concentrations, more chromium ions are left unabsorbed in the solution due to the saturation of binding sites [110]. The study done by [142] confirms that an increase in metal concentration, from 100 mg/L to 500 mg/L, led to a decrease in percentage Cr(VI) removal (from 99.88% to 83.69%). But analysis of total Cr showed that uptake capacity of the biosorbent SAR1 increased (6.27 mg/g to 28.95 mg/g) with an increase in Cr(VI) concentration.

4.5. The Influence of Biosorbent Dosage. The increase in the biosorbent concentration favors the heavy metal biosorption because of the increase in surface area [150]. According to [167], Cr(VI) removal efficiency by microalgae *Scenedesmus* species increased rapidly from 45.9 to 92.9% with the increase of S/L from 2 to 10%(w/v), and on further increase up to30% (w/v) is slightly increased to 96%.

4.6. The Influence of Agitation Speed. Agitation enhances dissolved oxygen or aeration which in turn strongly favors microbial growth and hence increases chromate reduction. However, there may be mechanical damage to microbial cells as a result of high agitation speed [88].

Generally, pH, temperature, contact time, stirring speed, and adsorbent dosages are the major factors that highly influence the removal efficiency of chromium ions from the solutions. This means that efficient and maximum biosorption can take place at specified temperature, pH, contact time, and dosage of adsorbent depending on the specific types of microbial biomass used.

5. Mechanisms of Chromium Biosorption by Microorganisms

Microbes are organisms that are capable of tolerating unfavorable circumstances, and these mechanisms evolved for the past millions of years. There are several protection mechanisms of heavy metal resistance by microbial cells. These mechanisms are an extracellular barrier, extracellular sequestration, and active transport of metal ions (efflux), intracellular sequestration, and reduction of metal ions [9]. The biological process of heavy metal removal can either be biosorption or bioaccumulation according to the cell's metabolism process [183, 184]. The two-bioremediation process differs mainly by the nature of sorbent, which in this case is the material of biological origin known as biosorbent. Bioaccumulation is a metabolism-dependent process in which the intracellular uptake of metal occurs due to increased membrane permeability. It can occur only with living organisms in which the contaminants are transported into the cell, and the metal ions are accumulated inside the cell of the biosorbent [183–185]. Biosorption, on other hand, is a quick, independent, and metabolically passive process responsible for the selective sequestration of heavy metal ions by dead/inactive biomaterials [183, 184]. During biosorption, the heavy metals bind to the cell walls extracellularly, while they bound to the proteins such as metallothionein once the metal ions are inside the cells in the bioaccumulation process.

The biosorption process always involves a solid phase which serves as the biosorbent (various biological materials). Due to the higher affinity of the sorbent for the sorbate species, the sorbate is attracted and bound thereby various mechanisms [181]. Biosorption is a nondirected physiochemical interaction that occurs between metal species and the cellular components of biological species. The mechanisms behind their resistance include adsorption, uptake, methylation, oxidation, and reduction of toxic, highly soluble Cr(VI) to less soluble and less toxic Cr(III) [186]. Heavy metal ions can be entrapped in the cellular structure of such organisms and subsequently be absorbed onto binding sites present on it (Figure 6). Biosorbents contain a variety of functional groups, including carboxyl, imadizole, sulfhydril, amino, phosphates, sulfate, thioether, phenol, carbonyl, amide, and hydroxyl moieties [181].

Microorganisms interact with metal ions through cell wall-associated metals, intracellular accumulation, metal siderophore, extracellular polymeric reactions with transformation, extracellular mobilization or immobilization of metal ions, and volatilization of metals [15]. As biosorption is a process in which physicochemical interaction between the charged surface groups of microorganisms and ions in the solution takes place by the process of complexation, ion-exchange, microprecipitation, adsorption [176, 187]. Metal sequestration or uptake in the case of bioaccumulation is followed by a number of processes such as localization of metal (Cr) within cell components, metallothionein binding, metal accumulation, extracellular precipitation, and complex formation [88]. Microbial Cr(VI) removal typically involves three stages: binding of chromium to the cell surface, translocation of chromium into the cell, and reduction of Cr(VI) to Cr(III). In general, Cr(VI) reduction by microorganisms may proceed on the cell surface, outside the cell, or intracellularly, either directly via chromate reductase enzymes, or indirectly via metabolite reduction of Cr(VI) [188].

6. Functional Groups Involved in Microorganisms during Biosorption of Chromium

The functional group is one of the major determinant factors in studying the removal of heavy metals from a contaminated environment using biological methods. Identifying functional groups responsible for the binding metal ions to the microbial biomass is very helpful in determining the biosorption mechanism and also important to decide on the plausibility of biomass for the process. Biosorbent containing multiple functional groups are more plausible for biosorption as the presence of multiple binding sites widens more contaminants binding opportunities. The type, structure, and connectivity of functional groups may considerably vary from microorganism to microorganisms and most of these groups have been characterized mainly on the microbial cell walls. The functional groups such as aldehydes, alkyl chains, amide, amine, alcohols/phenols, carboxylic, ester, organic halide compounds, phosphate, sulfoxide, and aliphatic organic chains of cellulose were identified as functional groups for the biosorption of chromium [7].

According to Javanbakht et al. [189]; the microbial biomass with oxygen (O–), nitrogen (N–), sulfur (S–), and/phosphorus (P–) containing functional groups directly participate in the binding of certain metal ions. The active functional groups present in microbial cells that are responsible for metal ion binding and the adsorption isotherm followed is given in Table 2. Different spectroscopic and microscopic techniques, such as infrared and Raman spectroscopy, electron dispersive spectroscopy, X-ray photoelectron spectroscopy, nuclear magnetic resonance (NMR), X-ray diffraction analysis, X-ray absorption fine structure spectroscopy, scanning electron microscopy (SEM), and transmission electron microscopy (TEM), have been employed for identifying some active sites involved in binding of heavy metal ions [189, 201]. However, most studies employ Fourier-transform infrared spectroscopic (FT-IR) technique to identify and characterize certain functional groups present in microbial biomass for uptake of toxic heavy metals such as hexavalent chromium. FTIR

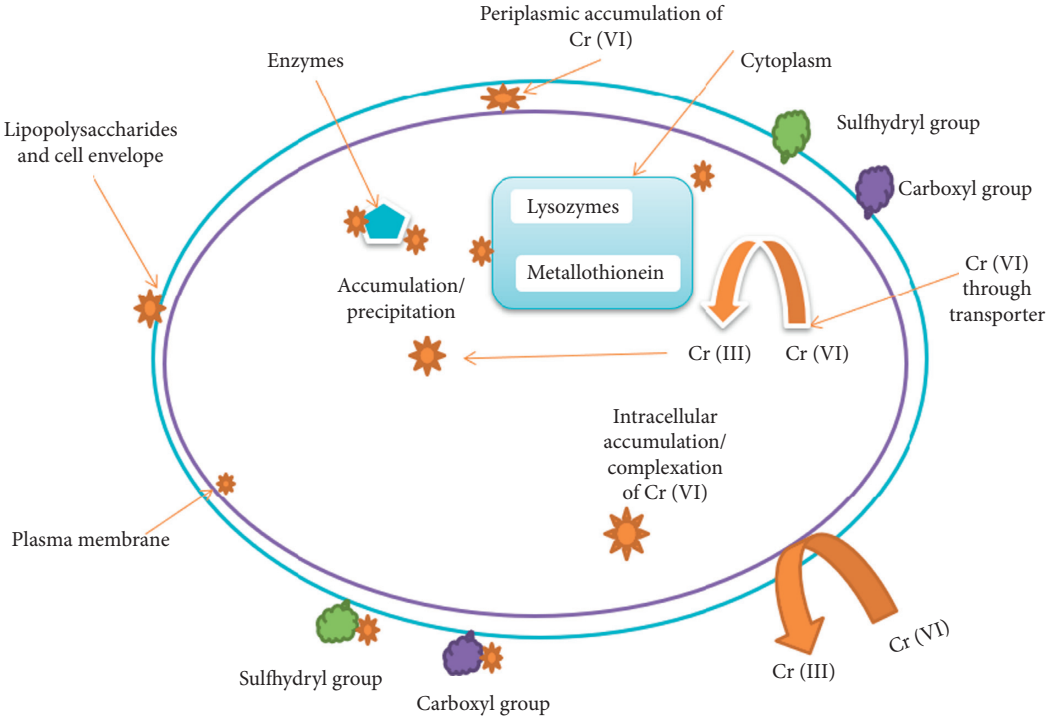

Figure 6: Mechanisms for Cr(VI) biosorption by microorganisms [88], [103].

Table 2: Involvement of various functional groups as revealed by FTIR analysis in chromium binding by different microorganisms.

S/no	Fungi	Functional groups	Adsorption isotherms	References
		FUNGI		
1	*Aspergillus foetidus*	C=O, C-Cl, PO_4^{-3} amine, N=C=S, OH, C-O	Freundlich	[190]
2	*Aspergillus niger*	C=O, amine, C-OH, C-NH_3	Freundlich	
3	*Aureobasidium pullulans*	C=O, C-H, amine, N-N=O, C=C-H	Freundlich	
4	*Aspergillus terricola*	C=C-H, C=O, C-OH, amine, C=N	Freundlich	
5	*Cladosporium resinae*	C=C-H, COH, C=C-H C=C, N-N=O, CS-NH, C=S	Freundlich	
6	*Acremonium strictum*	C=O, CH_3, C-NH_3, C=C-H, C-Cl, C=N, C-O, COH	Langmuir	
7	*Phanerochaete chrysosporium*	C-O, C=C, C-Cl, amine, C=O, C=N, CS-NH, PO_4^{-3}	Freundlich	
8	*Paecilomyces variotii*	Amine, CC, CN, OH, PO_4^{-3}, C=S, OH	Langmuir	
9	*Aspergillus terreus*	-OH, -CH, N-H, -SO_2-N, P-O	—	[151]
10	*Pleurotus ostreatus*	NH and COOH	—	[153]
11	*Aspergillus niger*	-COOH, -OH, -NH_2	—	[187]
12	*Arthrinium malaysianum*	–OH, C–O, C=O, –NO_2, C_xOH	—	[191]
		BACTERIA		
1	*Bacillus marisflavi* and *Arthrobacter* sp	_OH, –NH acetamido group, amide bond, C=O of COO-, free phosphates, phosphate groups, –CN	—	[192]
2	*Klebsiella* sp.	-NH2, O-H, -CONH-, -COOH, C = C, -CH_2	Freundlich	[141]
3	*Halomonas* sp. DK4	–OH, –CH_2, N-H, P–O–C, C=O	Langmuir	[140]
4	*Bacillus cereus* Pf-1	–OH, –CH and –NH stretching, C = O, –COO^-, –C–O and –C–N	Langmuir and Freundlich	[193]
5	*Sinorhizobium* sp. SAR1	N-H, O-H, C-O, P-O, C-H	Langmuir	[142]
6	*Pseudomonas aeruginosa* Rb-1 and *Ochrobactrum intermedium* Rb-2	-OH, -NH, S-H, C=C, carboxylic group, SO_2O^-, C-O, S = O, -C-C- and C-Cl,	—	[194]
7	*Bacillus cereus* IST105	O–H, N–H, C=C, P=O, C–O,	—	[195]
8	*Streptomyces werraensis* LD22	O–H or N–H, C–H, C–O	—	[196]
		MICROALGAE		
1	*Spirulina platensis*	C=O, C-C, C-O-C, P=O groups, COO-, CH_2 and NHC(O) amid groups, Carboxyl groups	—	[197]

Table 2: Continued.

S/no	Fungi	Functional groups	Adsorption isotherms	References
		FUNGI		
2	*Chlorella miniata*	Carboxylate group, C–O, amide I and amide II, amino group, O–H and N–H, $-CH_3$ and $\diagdown\!\diagup CH_2$, P=O and C–O	Langmuir	[198]
3	*Chlorella miniata*	O-H and N-H, C-H, $-CH_3$, COO-, P=O, C-O	—	[199]
4	*Spirulina platensis*	–OH and –NH, C=O, -C triple bond, C-H, carboxylate and phosphoryl groups	Freundlich	[161]
5	*Isochrysis galbana,*	–OH, –COOH and C=O, –NH,	Langmuir and Freundlich	[162]
6	*Pediastrum boryanum*	–OH, –COOH, –NH, C–O, $-CH_3$, C–OH, C–N–C,	—	[174]
7	*Lyngbya putealis*	-NH, -CH, -C≡N, and –C=C	—	[200]
8	*Scenedesmus* sp	N-H, O-H, C-H, -COOH, C-F, C-Cl, C-Br, C-O	Langmuir and Freundlich	[167]

spectroscopy analysis was carried out to obtain the characteristics of the functional groups and to identify the chemical bonds that played a significant role in the process of biosorption of chromium [141].

As a whole, the functional groups associated with the cell wall significantly vary from microorganism to microorganisms. In addition, existence of different functional groups such as amine, amide, carbonyl, and phosphate together in microbial cell widens the intracellular accumulation and extracellular adsorption of toxic heavy metal ions. Therefore, functional groups play pivotal roles in the removal of heavy metal ions from the solution and the mechanism associated can also be determined after knowing certain functional group involved in biosorption.

7. Conclusion

This paper reviews chromium biosorption by various microorganisms such as bacteria, fungi, and microalgae, as well as factors that influence metal removal and sorption mechanisms. Microbial remediation of Cr(VI) from the environment is one of the most viable and sustainable methods for reducing excess Cr(VI) levels in the environment. These microbes have evolved impressive mechanisms to preserve their homeostasis and resistance to heavy metals to thrive in such a toxic environment. The biosorption process is a microorganism-based technology that is cost-effective, safe, and simple to use for removing chromium from the water environment, and it has a lot of potential for future applications. Mechanisms involved in the biosorption process include transport across the cell membrane, complexation, ion exchange, precipitation, and physical adsorption. Metabolism-independent metal binding to the cell walls and external surfaces is the only mechanism present in the case of nonliving biomass. The biosorption capacity of biosorbents can be affected by the pH of the environment, temperature, contact time, biomass dosage, initial metal concentration, and other factors. Since, industrial wastewaters may contain more than one toxic heavy metal unlike laboratory solutions, simultaneous removal of many coexisting pollutants may be challenging. Hence, further research on these topics is needed to take full advantage of microbial biotechnology in environments. [201]

Data Availability

No data were used to support this study.

Conflicts of Interest

The authors declare they have no conflicts of interest.

Acknowledgments

The authors wish to acknowledge all who had been instrumental in the creation of this review article.

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