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Phytoreactor for Arsenic Biodetoxification: An Integrated Sequential Process of Phytoremediation Involving Multi-Kingdom Organisms

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ABSTRACT

Phytoremediation of polluted environments has received attention and developed rapidly in recent decades. Plant performance assessment is conducted around the effectiveness and efficiency of reducing the concentration of pollutants and certain plant-associated organisms. While supporting these advances, this paper aims to develop integrated phytoremediation as a pollutant treatment reactor involving all processes and multi-kingdoms of organisms, referred to as phytoreactor. The data collected comes from the results of previous studies related to various kinds of phytoremediation processes. Screening and selection of data were based on criteria for differences in plant processes, involvement of various plant-associated organisms, and aesthetics. An arsenic pollutant, one of the most toxic metalloids and ubiquitous, the kingdom's involvement of plant-associated organisms between aboveground and belowground plant parts. A new perspective in phytoremediation is creating a phytoreactor that integrates three sequential processes. Starting with the containment of toxic pollutants, followed by a primary process consisting of physicochemical and biological processes, and completed by a secondary process in plants, which produces nontoxic environmental media conditions. The primary biological processes are carried out in the rhizosphere and phyllosphere. The involvement of the plant-associated organism kingdom is different in the rhizosphere and phyllosphere due to the suitability of the habitat, the type of pollutant, and the aesthetics of the application of the phytoreactor. Phytoreactor for the remediation of polluted environments involves synergistic multi-kingdoms of plant-associated organisms for specific types of pollutants in the rhizosphere and phyllosphere.

1. Introduction

Plants coexist with all the kingdoms of organisms as environmental bioresources for human life. While the environment and humans emit various substances, plants and other organisms can control these emissions. At the level of a polluted environment, plants are seen as having the potential to remedy it through phytoremediation. Phytoremediation refers to the process by which plant communities remove pollutants in environmental media. Various physical, chemical, and biological

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pollutants can coexist in an ecosystem. In dealing with microbial pollutants, such as *Escherichia coli*, phytomicroremediation eliminates the pathogenic bacteria with plant-derived compounds having antiseptic properties [1]. Plants have long been known to produce many antiseptic secondary metabolites, such as phenolic compounds [2]. Myrtaceous plant species *Leptospermum scoparium* and *Kunzea robusta* release antiseptic compounds against pathogenic microbial communities in the soil ecosystem, especially the bacterium *E. coli* [3]. Thus, naturally, plants eliminate pollutants from the products of their own life.

During the phytoremediation process, plants must stay alive by meeting the needs of beneficial physical and chemical factors, such as sufficient water and nutrients. In addition, living plants need collaborative support with other organisms. For example, plants need the support of root bacteria to degrade organic matter into carbon dioxide, which is their essential need. On the other hand, there are phytopathogenic bacteria such as *Pseudomonas syringae* pv. *actinidiae*, a bacterium that causes cancer in kiwifruit [4]. The same is the presence of fungi, especially the phyla Ascomycota and Basidiomycota [5], which can be phytopathogenic [6]. However, these phytopathogenic fungi can be controlled by the fungal genus *Trichoderma* [7]. In short, healthy plants require beneficial cooperation with plant-associated organisms.

In practice, the growth of one healthy plant species in an ecosystem polluted by various pollutants may not eliminate all pollutants. A type of plant has a limited ability to remove pollutants from its environment. An example is the water hyacinth plant in a pond containing many pollutants, which results in different removal efficiencies, some of which do not reach safe concentrations [8]. To eliminate various pollutants, one can use various plants placed in polluted ecosystems [9]. With the use of one species or multispecies, plants need the support of other organisms to at least have the ability to recycle plant exudates. Thus, in the life cycle of healthy plants, the involvement of organisms is imperative, likewise, when plants are used to process pollutants.

Therefore, this literature review presents a new perspective on phytoremediation using synergistic collaborations across plant-associated organismal kingdoms for a particular type of pollutant. The clarity of the collaboration of various kingdoms of organisms can be a direction to improve the effectiveness of plants in performing various pollutant phytoremediation.

2. Methods

The centre of assessment is the plant with the interactions between the various kingdoms of the plant-associated organism within the phytosphere. Phytosphere does not have a specific size for area or volume, except for the environmental area affected by plant life processes. The phytosphere includes the rhizosphere and aerial parts including the stems and phylllosphere, both for aquatic and terrestrial plants.

The taxonomy of organisms follows the seven kingdoms [10]. The kingdom of organisms includes Plantae, Fungi, Animalia, Protozoa, and Chromista, all five eucaryotes containing a nucleus surrounded by a membrane. At the same time, it involves prokaryotic organisms that do not contain a membrane-bound nucleus, namely Eubacteria, referred to as Bacteria, and Archaea bacteria, from now on referred to as Archaea.

Each kingdom of plant-associated organisms should be able to eliminate the same pollutant directly or indirectly to confirm whether there is a collaboration between kingdoms. Inorganic pollutant arsenic (As) is considered due to listed as the most dangerous substance to human health [11]. The biological role of eliminating the same pollutant uses data from previous research in the last ten years.

The literature search used Harzing's Publish or Perish 8 software. Article searches were open-access articles indexed by PubMed and Crossref. At least three articles, which were pollutant-organism specific, were selected to reinforce the interactive relationship between the organismal kingdoms mutually.

3. Results and Discussion

The first section describes the plant as a pollutant processing reactor to identify the complete stages of the phytoremediation process. The second describes arsenic characterized by exposure to various environmental media. The third identifies the kingdom of plant-associated organisms capable of processing the pollutant in the rhizosphere and phyllosphere. In the end, the suitability of arsenic biodetoxification is summarised, along with proposed applications in indoor and outdoor environments.

3.1 Phytoreactor and Processes

Man-made conventional water and waste treatment reactors have linear processing stages [12,13]. The influent stream undergoes successive processes and ends as treated effluent [14-16]. In addition, the reactor is a non-living material, so it cannot protect itself from external disturbances [17]. Meanwhile, there is no doubt about the ability of plants to process pollutants in soil [18], water [19], and air [20]. Therefore, a plant can be seen as a pollutant processing reactor, after this referred to as phytoreactor.

The special advantage of phytoreactor is that plants are alive, can grow, and have the ability to protect their health [21]. Moreover, the influent stream can enter every main part of the plant: roots, stems, and leaves [22], so various forms of influent: solid, liquid, and gas, can be processed simultaneously. Even effluent streams can recycle themselves, such as influent organic matter converted to carbon dioxide, which is the life supply of plants. The power of the plants is promising for waste treatment applications and the phytoremediation of polluted environments.

Retrospective phytoremediation data processing for decades resulted in the formulation of the phytoreactor depicted in Figure 1. It is necessary to emphasize that phytoreactor is not just a plant process but an integrated flow of pollutant containment, primary physicochemical and biological processes, and secondary plant processes. The three integrated processes are balanced with phytomicroremediation, which releases antipathogenic plant-derived compounds to maintain plant health. Likewise, the remediation process without the presence of plants, which is commonly known as bioremediation. Shortly, phytoreactor involve bioremediation and not vice versa.

Figure 1 describes, firstly, in fulfilling the need for water for plant life, plants undergo the process of transpiration of water from their growth medium. The water flow simultaneously immobilizes pollutants in the rhizosphere. This immobilization process is called phytostabilization [23] and contains pollutants in the rhizosphere.

At the same time, plants absorb carbon dioxide and water in the photosynthesis process for biomass growth and constantly the respiration process. The two processes of photosynthesis and respiration are also followed by the transfer of gaseous pollutants, which are transported through the stomata. Stomata are the tiny pores of plants for breathing and are located in all parts of the plant, especially on the upper and lower sides of leaves, flower petals, stems, and roots [24]. Thus, the whole plant can be considered a containment body of gaseous pollutants, called phytosequestration [25]. Furthermore, phytosequestration in roots emphasizes pollutant uptake through phytochemical exudation and in roots through mechanisms of protein transfer and cellular processes [26].

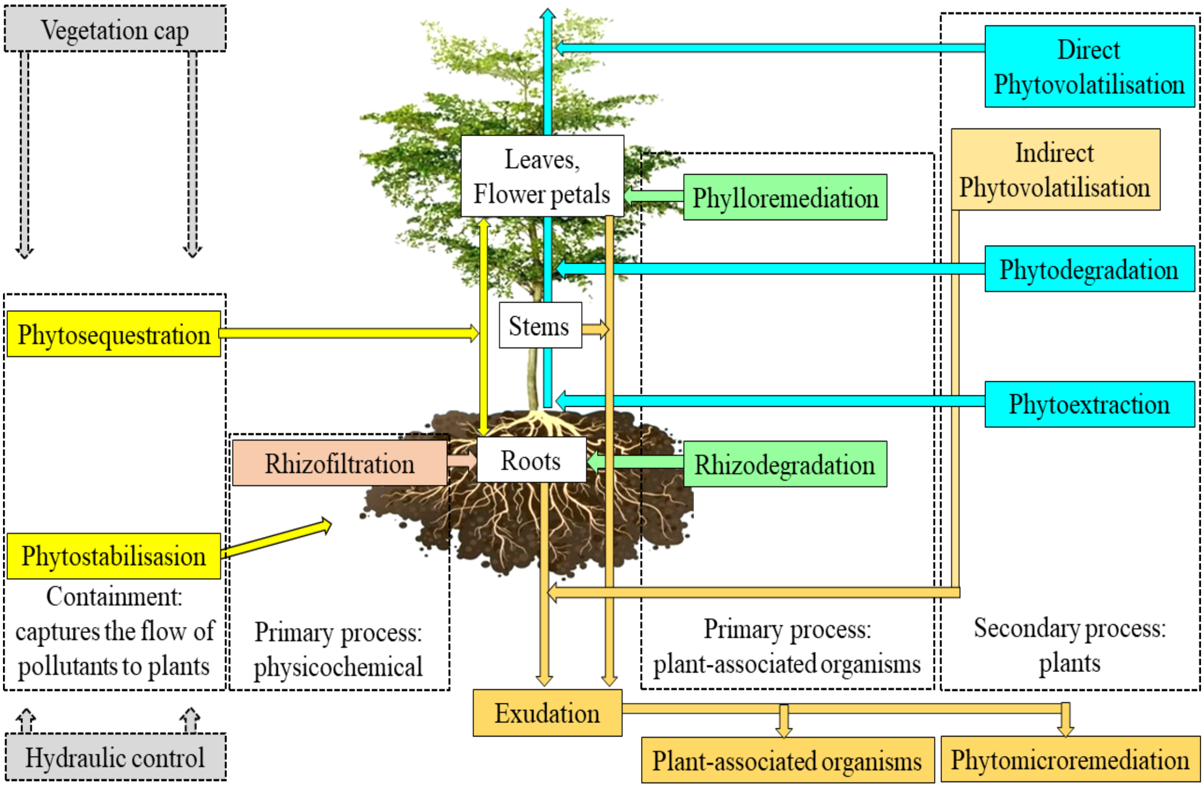

Fig. 1. Phytoreactor processes for removing pollutants

In the leaf zone, there is microbial life known as leaf-associated microbes. The microbial processes can occur for aerosols, which consist of liquid and solid particles and are referred to phylloremediation [27]. This process can be considered the primary process of pollutants to be further processed in various ways by plants.

Aerosols can be exposed to growing media, water, or soil [28]. Precipitated aerosols and immobilized pollutants can undergo various processes in the rhizosphere before being absorbed into the plant for further processing. Various physical, chemical, and microbial processes can operate simultaneously and sequentially within the rhizosphere. Physical-chemical processes such as precipitation, adsorption, and filtration on roots are known as rhizofiltration [29]. For pollutants that soil microbes can convert, the process is called rhizodegradation [30]. The two rhizosphere processes can also be seen as a primary process in phytoreactor.

Pollutants resulting from the rhizosphere process then enter the plant, referred to as phytoextraction [31]. All pollutants that enter the plant, either from direct phytosequestration or from the results of rhizosphere processes, undergo a degradation process known as phytodegradation [32]. Volatile pollutants in plants can experience release outside the plant, referred to as direct phytovolatilization via stems and leaves [33]. The sequence of these three processes is a secondary process from the plant's point of view as a reactor for processing environmental pollutants.

Outside the processing of pollutants by plants but balancing the remediation process, as living organisms, plants secrete metabolites as exudates through various plant organs such as leaves, stems, and roots [34]. This exudation is the reverse of phytosequestration by releasing metabolites into the environment or indirect phytovolatilization via roots [33]. Exudate release into the rhizosphere is beneficial, partly for supplying organic matter for plant-associated organisms and partly for phytomicroremediation.

During rainy seasons, the plant canopy becomes a vegetation cap [26] to reduce rainwater falling within the phytosphere. As a result, the pollutants concentrated in the rhizosphere are not dispersed due to the effects of rain.

Under certain conditions where the phytoreactor area has a deep groundwater level, plants can raise the water level close to the rhizosphere. Such events demonstrate the ability of plants to control groundwater hydraulically [26].

3.2 Arsenic Exposure and Attenuation

In the outdoor environment, the source of arsenic is the earth's crust, which can be released into the soils, waters, and air through natural and human activities [35]. Naturally, the main mineral arsenic binds to sulfides in forms such as realgar (AsS), dimorphite (As_4S_3), and uzonite (As_4S_5). Due to chemical-physical environmental factors, natural minerals, and their use can change to become the polymorphs of arsenolite and claudetite (As_2O_3) [36].

Arsenic is a metalloid substance that forms toxic compounds. The trivalent arsenite is more toxic than oxidized pentavalent arsenate [37]. The closest places to poison arsenic are soil and water environments. Inorganic arsenic is easily soluble in water and acutely toxic to human health, which can lead to chronic arsenicosis [38]. Meanwhile, arsenic exposed to flora and fauna forms organic arsenic compounds, such as arsenobetaine (AsB) [39]. The bodies of environmental organisms easily eliminate these organic compounds so they are less toxic to human health.

In indoor environments, one source is using paints containing arsenic pigments [40]. In the short term, arsenic concentrations may not harm living things. However, changes in physical and chemical environmental factors in the long term can threaten indoor life. The air humidity factor affects the accumulation of arsenic, which increases with room humidity. The problem occurs in residential areas, where humidifiers filled with tap water produce arsenic concentrations four times higher than the arsenic standard in drinking water [41]. The result explains that arsenic is a metalloid substance that dissolves easily in water.

Where indoor decorative plants are found, it is beneficial to indoor air quality [42]. Like the ability of outdoor flora above, indoor plants biotransform arsenic to be less toxic. The path of exposure to arsenic in the environment and its attenuation with simplification related to phytoreactor can be depicted in Figure 2.

The strategic area for arsenic detoxification covers the rhizosphere and phyllosphere. In both areas, arsenic undergoes bioremediation through rhizodegradation and phyloremediation. Thus, the phytoreactor output is less toxic arsenic in plants and the surrounding environment.

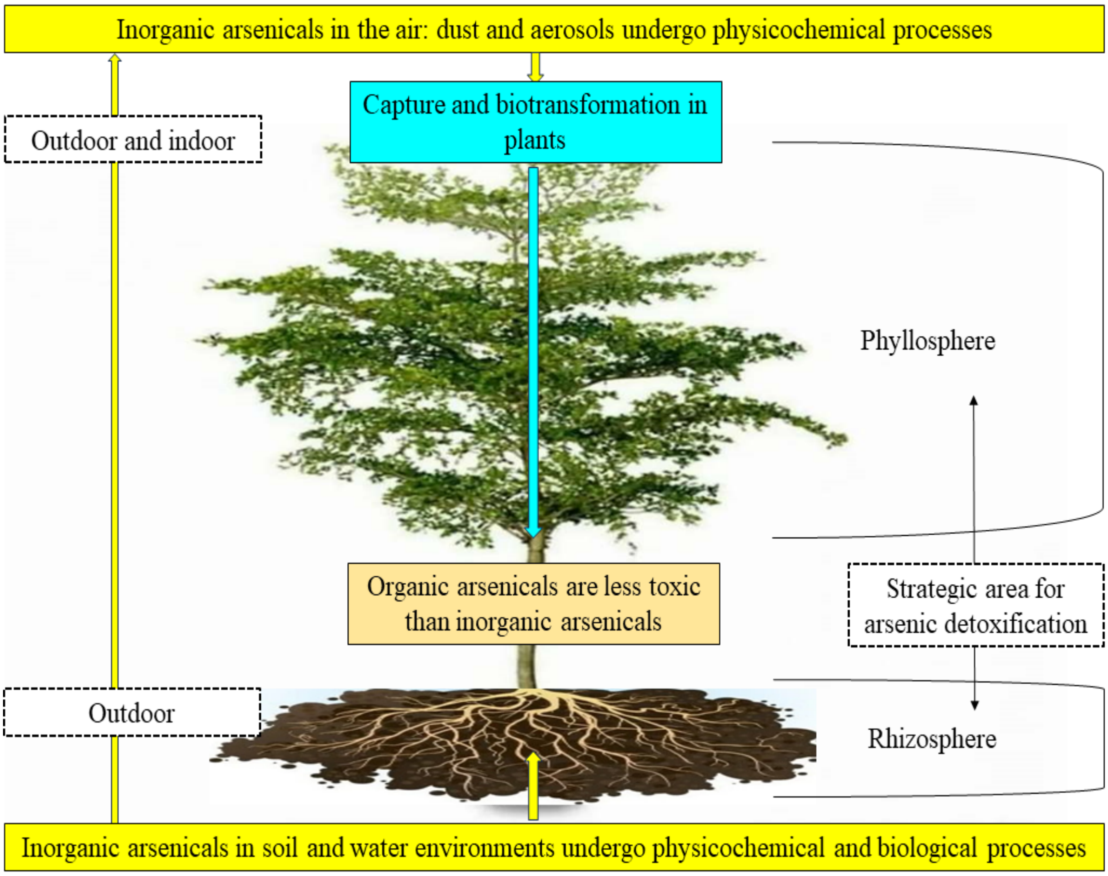

Fig. 2. Transport and transformation of arsenic related to phytoreactor

3.3 Rhizodegradation Detoxifies Arsenic

The biotic richness of the soil ecosystem, apart from terrestrial plants, includes various kingdoms of organisms. In the soil can live eukaryotes Fungi, such as *Trichoderma* sp. [43]; Animalia, such as earthworm *Eisenia fetida* [44]; Protozoa, such as *Colpoda* sp. [45]; and Chromista, such as Oomycetes [46]. Bacteria Actinomycetes [39] and Archaea Nitrososphaerales [43] are abundant in prokaryotes. Likewise, in water ecosystems, aquatic plants can live together with various kingdoms of eukaryotes Fungi [47], aquatic earthworms Oligochaetes [48], Protozoa [49], Chromista [50], and both prokaryotes are ubiquitous [51] that they are undoubtedly present in water.

Fungal taxa are abundant in healthy-looking trees. On the roots, the richness of the fungal species is *Trichocladium griseum*, and *Penicillium restrictum* [52]. A direct symbiotic association between plant roots and Fungi forms mycorrhizae [53]. The potential of mycorrhizae to control arsenic exposure to plants has been studied, which results in arsenic accumulation in soil [54]. Mycorrhizae increased cysteine, glutathione, and non-protein thiols and can immobilize arsenic and transform it into nontoxic complexation [55]. Therefore, mycorrhizae become an important functional group for eliminating inorganic arsenic and producing organic arsenic.

Another biological relationship between Fungi and green algae forms lichens [56]. The epiphytic lichen *Xanthoria parietina* is physiologically susceptible to exposure to arsenic up to 0.01 ppm [57]. Likewise, in the community of lichens-associated organisms, Bacteria can accumulate without transforming inorganic arsenic [58]. However, other lichens species of *Heterodermia diademata* (Taylor) D.D. Awasthi, *Phaeophyscia hispidula* (Ach.) Essl., *Usnea longissima* Ach., *Roccella montageni* Bél., *Parmotrema tinctorum* (Despr. ex Nyl.) Hale and *Sticta* sp. were reported can decrease the arsenic concentration. Species of *Heterodermia diademata* having the highest elemental contents of

Carbon, Nitrogen, Hydrogen, and Oxygen is the most effective in removing arsenic of about 40 ppm [59].

Earthworms can reduce the bioavailability of heavy metals [60]. Several species of earthworms, such as *Eisenia fetida*, *E. andrei*, *Perionyx excavatus*, *Eudrilus eugeniae*, and *Lumbricus rubellus*, transform arsenic [61]. Accumulation of metals in the body of earthworms induces metallothioneins proteins to bind metals [62]. Even the earthworm species *Lumbricus rubellus* possesses surface-sensitive drilodefensin molecules, which counteract the toxicity of plant-derived polyphenols [63]. A recent study found a new nematode species, *Tokorhabditis tufae*, capable of living in arsenic-rich environments [64]. However, a species of the nematode phylum, *Caenorhabditis elegans*, showed differences in organic and inorganic arsenic toxicity effects. The dimethylarsinic acid metabolite of the species is even more toxic than inorganic arsenic for the third generation onwards [65].

The freshwater Protozoa Tetrahymena pyriformis was reported to be able to transform inorganic arsenic into organic arsenic dimethylarsenate with sufficient phosphate concentration. In the phosphate concentration range of 3-30 ppm, the dimethylarsenate product is linear, increasing with the phosphate content [66].

Chromista organisms, brown algae Oomycetes [67], are plant parasitic pathogens [68]. However, the organism can detoxify heavy metals through extracellular immobilization and intracellular biosorption, which exceeds the ability of bacteria [69,70]. One can choose plant species capable of working as phytomicroremediation in dealing with Oomycetes because other species of Chromista can accumulate arsenic [71].

Bacteria capable of reducing arsenate are ubiquitous in soil and water contaminated with arsenic, such as *Anaeromyxobacter dehalogenans* found in soil [72]. In addition, a species of *Pseudomonas aeruginosa* is resistant to exposure to arsenic. Concentrations up to 1.4 g/L arsenite and 7 g/L arsenate, can remove more than 90% of these pollutants [73].

Archaea diversity is high in soil habitats, especially in the rhizosphere [74]. However, Archaea are much less understood in dealing with arsenic than Bacteria, and fewer genomes have been sequenced [75]. Moreover, arsenite removal is suggested using mixed microbial oxidation, such as iron and manganese oxidizing bacteria [76]. At least, it was found for halophilic Archaea *Haloarcula* sp. IRU1 capable of immobilizing arsenite. The yield of arsenite bioaccumulation reaches about 60% at a temperature of 40^{o}C and a pH of 8 as a determinant [77].

3.4 Phylloremediation Detoxifies Arsenic

From the point of view of area coverage, phylloremediation is promising for overcoming the problem of air pollution. At least for plants with a single leaf layer for a given land cover area, the upper and lower leaves' total surface area exceeds the soil covered by the plant canopy. The total leaf area coverage can continue to increase as the plant grows and regenerates.

On the leaves, the richness of the fungal species is *Trichomerium* sp. 5238_8, *Aureobasidium pullulans*, *Cladosporium* sp. 5238_5, and *Vishniacozyma carnescens* [52]. The fungal species in the leaves are more diverse than the root fungi. In particular, endophytic fungi in the leaves increase the bioactive compounds for several medicinal plants in Nigeria, such as *Acalypha 27ifurc, Albizia zygia, Alcharnea cordifolia,* and *Chrysophyllum albidum* [78]. In addition, lichens containing fungi, such as *Evernia mesomorpha, Flavoparmelia caperata,* and *Physcia aipolia/stellaris* are useful and reliable for air pollution indicators [79].

Terrestrial ecosystems are rich in organisms, and all kingdoms of organisms have been shown to play a beneficial role in arsenic detoxification without causing harmful effects to plants. In addition, all the organisms referred to live in a position that does not damage the aesthetics from a human point of view. However, not all kingdom organisms can be considered for their benefit in detoxifying arsenic in the phyllosphere. For example, nematodes living on leaves are mostly pathogenic to plants, such as the Aphelenchoides genus and common species of Aphelenchoides (*A. besseyi, A. bicaudatus, A. fragariae, A. ritzemabosi*) [80]. Another species of *Caenorhabditis elegans* can grow in the phyllosphere, especially in decomposing fruits and stems [81]. Low arsenite concentrations can make the nematode-resistant to heat and chemicals and extend its life span [82]. These results indicate that arsenite can be accumulated by the nematode in question but is not considered a useful animal in phylloremediation.

Some protozoa, such as amoebas and ciliates can ingest pathogenic bacteria and package them in their membranes. The encapsulated bacteria become more resistant to environmental stress and survive than unpackaged bacteria [83]. Meanwhile, the bactivorous protozoa, *Colpoda steinii*, did not affect the abundance of the kingdoms Bacteria and Archaea and the phyla Proteobacteria and Bacteroidetes. The effect of abundance shifts occurs at the bacterial species level. The influence of protozoa targets certain bacterial species, or certain bacterial species can avoid protozoa predators [84].

The brown alga Chromista was found to accumulate arsenic [85], but its ability to transform arsenic is unknown. However, the organism has the potential to immobilize arsenic from the environment. Even the species brown algae of *Bifurcaria 28ifurcate,* and *Fucus spiralis* can be used as fertilizer to enhance plant growth [86].

Most phyllosphere organisms are non-pathogenic Bacteria. The abundance depends on plant species, diversity of organisms, and physical-chemical environmental factors [87]. Some of these are numerous duckweed leaf-associated bacteria, which can oxidize toxic arsenite to less toxic arsenate [88].

Archaea were found to be able to oxidize arsenite as bacteria do [88]. Their interactions with bacteria potentially increase plant growth through the biosynthesis of phytohormones, fixation of nutrient carbon and nitrogen, and protection against abiotic stresses [89].

3.5 Suitability of Arsenic Biodetoxification

Based on the interaction of arsenic and the environment, the exposure strategy is containment within the rhizosphere. For arsenic elimination, the strategy is to maximize the conversion of trivalent arsenite to oxidized pentavalent arsenate and less toxic organic forms. These strategies use a diverse approach to the kingdom of organisms for terrestrial and aquatic plants. External chemicals can be added sufficiently to optimize process conditions. A summary of arsenic biodetoxification by the diversity of the organismal kingdoms is depicted in Figure 3.

Figure 3 shows the suitability of the phytoreactor application in the field. In operations, phytoreactors require priority treatment to maintain plant health, followed by treatment to eliminate Animalia in the phyllosphere for aesthetic purposes. Organisms that are pollution indicators, such as lichens, can be used for monitoring purposes.

One way to maximize the detoxification of polluted air is to choose plant species with the widest possible leaf surface. Layered leaves characterize suitable plant species, and the size of each leaf is narrow [90]; an example is the evergreen plant. The successful removal of arsenic by evergreen plants has been demonstrated by the accumulation of arsenic in shoots of *Equisetum* spp. and *Calamagrostis epigejos* [91].

Selecting plant species with long roots that exceed the height of the aerial parts is suitable for detoxifying polluted soil and water and designing riparian zones (83). Examples of plants for soil and water remediation include vetiver. Vetiver plants remediated arsenic-polluted water and sediment with more than 90% and 80% removal efficiencies, respectively [92].

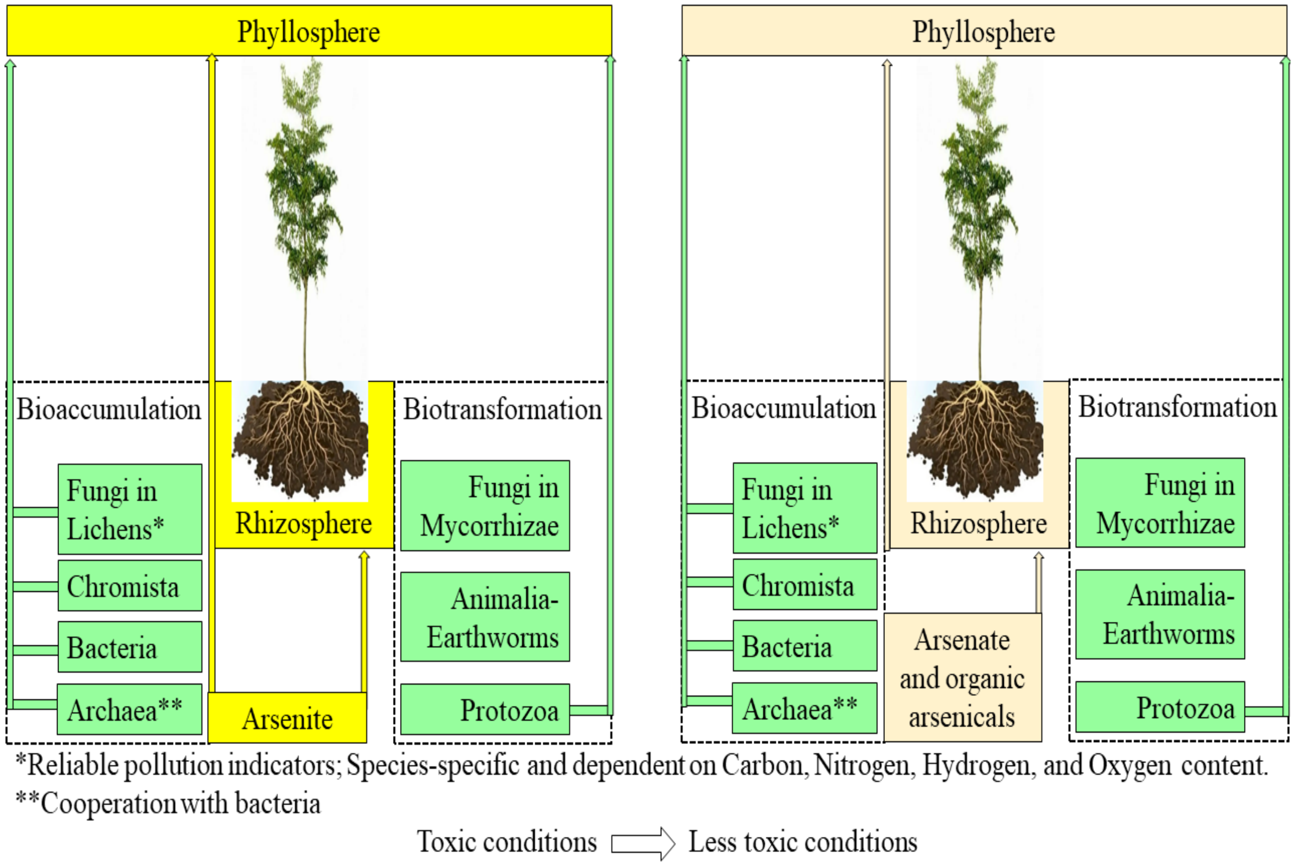

Fig. 3. Arsenic biodetoxification in the phytoreactor

In practice, pollutants can transport between environmental media, as shown in Figure 2, which directs the need for phytoreactors to cover all media. Therefore, plant diversity can meet the targets of all environmental media [93]. Ideally, it is necessary to grow plant species in a polluted area, some with long roots and some with narrow layered leaves.

All of the above uses imply the involvement of all organisms across kingdoms in the primary treatment of the rhizosphere and phyllosphere simultaneously. Each organism has a specific role, namely accumulation or transformation, in removing toxic inorganic arsenic into less toxic products. The differences in the variety of organisms and their specific roles direct the suitability of applying phytoreactors for the remediation of polluted environments and waste treatment. In particular, the specific accumulation ability makes organisms useful as bioindicators in operational and environmental monitoring.

4. Conclusion

Phytoreactor is a complex process involving synergistic cooperation between kingdoms of organisms and environmental factors. However, the phytoreactor processes can be simplified into three consecutive processes. The first is containment, where environmental pollutants flow toward the plant. At this containment stage, it is considered that there is no contribution of organisms, which deconcentrate and convert pollutants, except through chemical treatment. Thus, this containment stage is referred to as a physicochemical process. In addition, the primary process for pollutant detoxification involves organisms outside the plant kingdom. Therefore, this primary treatment can be referred to as a biological process. Lastly is the secondary treatment for further pollutant detoxification within the plant resulting in a treated flow to the environment. This stage is the ability of plants to process pollutants before releasing them into the outside environment. Hence, phytoreactor is an integrated sequential process of pollutant containment, physicochemical remediation, bioremediation, and plant process.

For the arsenic-polluted rhizosphere, all organism kingdoms are involved in rhizodegradation but not in phyllosphere. These differences challenge further in-depth research for the phytoreactor of various pollutants and their mixtures. So far, no research reports have been found regarding the ability of stems. When stomata are present in all parts of a plant, it is necessary to deepen research on the ability of stems to carry out remediation. Stems are above ground so that they can cover more air pollutants.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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