Chapter 12

Phytoremediation: Halophytes as Promising Heavy Metal Hyperaccumulators

Kamal Usman, Mohammad A. Al-Ghouti and
Mohammed H. Abu-Dieyeh

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Abstract

The continued accumulation of trace and heavy metals in the environment presents a significant danger to biota health, including humans, which is undoubtedly undermining global environmental sustainability initiatives. Consequently, the need for efficient remediation technologies becomes imperative. Phytoremediation is one of the most viable options in this regard. Hundreds of plants in laboratory experiments demonstrate the potential to remediate varying concentrations of heavy metals; however, the remediation capacity of most of these plants proved unsatisfactory under field conditions. The identification and selection of plants with higher metal uptake capacity or hyperaccumulators are one of the limitations of this technology. Additionally, the mechanism of heavy metal uptake by plants remains to be sufficiently documented. The halophyte plants are famous for their adaptation to harsh environmental conditions, and hence could be the most suitable candidates for heavy metal hyperaccumulation. The state of Qatar in the Gulf region encompasses rich resources of halophytes that have the potential for future investment toward human and environmental health. This chapter, therefore, gives an overview of phytoremediation, with emphasis on halophytes as suitable heavy metal hyperaccumulators for improved remediation of heavy metal–contaminated areas.

Keywords: halophytes, phytoremediation, heavy metals, hyperaccumulation

1. Introduction

Heavy metals and other organic compounds constitutes the major environmental contaminants, and the trials of phytoremediation to free pollutants from waste water and contaminated soil dates back to hundreds of years ago in plants such as the *Thlaspi caerulescens* and *Viola calaminaria,* which were reported to remediate high concentration of heavy metals [1]. Anthropogenic activities arising from industrialization largely contribute to the proliferation of these contaminants, either by direct leakage or accidents during transport of solid and liquid wastes from storage and industrial facilities [2, 3]. Strategies to clean up environmental contaminants, both organic and inorganic are either by physical, chemical and or biological treatments [4, 5]. However, physical and chemical methods are recognized for a number of disadvantages or limitations such as high cost and labor intensiveness. Additionally, chemical processes create another pollution and are especially costly since they generate heaps of sludge [6]. In view of this context, new and better approaches to clean up of metal contamination were thought up and became imperative, hence the exploration of various bio-based techniques. The use of biological agents is considered cheap, safer and has limited or no negative impact to the environment [7]. Bio-based remediation methods include bio-augmentation, bioremediation, bioventing, composting and phytoremediation. However, phytoremediation proves the most viable and useful alternative and has gain an increasing attention in recent times [8, 9]. The adverse and negative effects associated with these elements make them targets for phytoremediation [10]. Phytoremediation offer several advantages. It is cheap, promotes biodiversity, reduces erosion, less destructive and decreased energy consumption leading to reduced carbon dioxide emission [11]. To date, about 400 plant species were suggested to be metal hyper-accumulators [12]. However, few studies reported the toxicity of several metals combined [13], and while hyper-accumulation of nickel (Ni), cadmium (Cd), manganese (Mn), zinc (Zn) and selenium (Se) have been well established, the same is yet be available or demonstrated beyond doubt in plant species for copper (Cu), chromium (Cr), lead (Pb), thallium (Th) and cobalt (Co) metals. For instance, Cu is an important element for growth and general plant physiology, owing to its role as a cofactor to various types of enzymes involved in the transfer of electrons during metabolic processes, such as the tricarboxylic acid (TCA) cycle [14, 15]. However, at high concentrations, it is toxic to plants signaled by stunted growth, and although there is some physiological insight to Cu stress in plants, the responses are still vague at the functional level [16]. The accumulation of heavy metals in plant tissues results in a wide range of negative effects on growth. Although it affects seed germination, growth of seedlings and photosynthetic processes, which generally leads to the inhibition of the plants important enzymatic activity [17, 18], however, plants responds differently [19]. In dealing with the heavy metal stress, the root tissue is the first to be exposed to the associated toxins, and its cell wall has a mechanism of exchange that fixes the heavy metal ions, thereby limiting the transmission of the toxins to other plant tissues [20, 21]. Several studies reported many plants, including desert species as good phytoremediation agents, however, few are metal hyperaccumulators and their selection for efficient phytoremediation is still a challenge. This is demonstrated by slow growth, above ground biomass, root system and harvest [22]. Accordingly, successful heavy metal phytoremediation requirement of hyperaccumulation capacity in candidate plants position halophytes as suitable phytoremediators. This is

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due to their extensive stress tolerance mechanism, which enables them thrive in saline soil and in other desert conditions.

2. Phytoremediation

In simple terms, phytoremediation refers to a process where plants are employed to reduce or free up organic and inorganic contaminants from the environment [13] with the aid of associated microbes. The process by which contaminants are remediated differs; these may be in the form of removal, transfer, degradation and immobilization from either soil or water [23]. It is a unique approach capitalizing on plants roots ability for the initial uptake of pollutants, and eventually accumulating them onto the shoot tissue by translocation across the stem. Compared to other conventional treatment techniques, phytoremediation is new, with a great potential to providing the much-needed green technology solution to our deteriorating environment. To date, hundreds of plant species were suggested as potential phytoremediation agents [24].

2.1. Phytoremediation techniques

During phytoremediation, plants growing on soil or water contaminated with trace or heavy metals could absorb or tolerate these elements differently, depending on the physiological means involved and the kinds of metals present [25]. According to Halder and Ghosh [26] phytoremediation techniques are categorized into five; phytoextraction, phytofiltration, phytovolatilization, phytostabilization and phytotransformation.

2.1.1. Phytoextraction

Phytoextraction is a technique of phytoremediation where plants take up metals by translocation, and accumulate them in a form that can be extracted on its tissue [27]. It is one of the most common types of phytoremediation and the names; phytoabsorption, phytoaccumulation and phytosequestration are often used interchangeably to refer to phytoextraction [28]. It is considered as the major phytoremediation technique among all others for the removal of metals from contaminated water, sediment and soil. The efficiency of this remediation process depends on a number of factors from soil properties, metal bioavailability and speciation to the type of plant species. However, high concentration of absorbed metals usually ends up in the shoot biomass of the plant in harvestable form [12]. A number of recent studies reported various plant species that demonstrate phytoextraction strategy from both water and soil media [29–32].

Plants able to exhibit phytoextraction strategy in metal sequestration may potentially be hyper accumulators, referring to plants that consistently accumulate certain threshold of metal concentration in their shoot tissue, which varies according to the metals [22]. Generally, all hyper accumulators should possess characteristics such as high growth rate, widely branched shoot, high bioaccumulation and translocation capacity, high above ground biomass, easily cultivated and harvested [22, 33]. However, Ali, Khan [28] demonstrated two methods or approaches for metal phytoextraction in different plants, one producing less above ground biomass but significantly accumulate metals in high concentration and vice versa in the other plant species, with final metal accumulation in agreement with those of hyper accumulators. Consequently, hyper accumulation is more important in phytoremediation than volume of biomass produced, and this suggest the use of hyper accumulators as more acceptable since it has advantages such as safe disposal, cheap process and easy handling [28].

2.1.2. Phytofiltration

Phytofiltration or rhizofiltration, as used interchangeably, refers to the absorption or adsorption of contaminants from surface wastewater by plant roots thereby preventing them from leaching to the underground water [34]. It is a type of phytoremediation technique that can be demonstrated *in situ* by directly growing plants in the polluted water body [24]. Although it is commonly applicable using aquatic plant species [35], there are suggestions that the process may be applied to terrestrial plants, which remediate metals to precipitate with the aid of microbes root bio filter [36]. Indeed, root exudates cause metal precipitation which alters the rhizosphere pH level [37]. Many terrestrial plants including grasses grown in a hydroponic culture were shown to effectively remove metals such via phytofiltration [38]. In the same study, Indian mustard was especially reported to accumulate higher fold of metal concentration far beyond the initial concentration, and the removal is by tissue specific adsorption mediated by root metal concentration.

Quite a number of studies have shown many species of aquatic macrophytes that demonstrate phytofiltration potential. While experimenting for phytoremediation under different water conditions polluted with heavy metals, Liao and Chang [39] found that *Eichhonia crassipes* absorb and accumulates metal contaminants, it has also exhibit high growth rate and increased biomass production and thus considered a good phytofiltration agent. This plant species absorb high concentrations of Pb, Ni, Zn and Cu which accumulates much higher in the root tissue than the shoot, suggesting the important role of fibrous and tap root system found in the plant, which is one of the key characteristics of potential phytofiltration agent. In a similar study, other aquatic plant species including *Salvinia herzogii*, *E. crassipes*, *Pistia stratiotes* and *Hydromistia stolonifera* were shown to absorb high concentration of Cd with *P. stratiotes* accumulating higher Cd concentration and exhibiting faster growth rate, a feature attributed to possible complimentary mechanism for the enhanced metal uptake [40]. Absorption of Cd in the root of all the plants relates to the added concentration. In another study by Thayaparan, Iqbal [41] also reported that *Azolla pinnata* have shown a great potential in the removal of high Pb concentration by phytofiltration from polluted water. As in phytoextraction, potential phytofiltration agents should tolerate high metal concentration, exhibit fast and high growth rate as well as above ground biomass, however, in contrast to phytoextraction, they are expected to show limited translocation capacity of absorbed metals from root to shoot tissues [24]. For efficient phytofiltration, this is an advantage over phytoextraction, since low translocation of contaminants means reduced contamination of other parts of the plant.

2.1.3. Phytostabilization

In this technique, pollutants are converted into a less toxic or bioavailable form by the continuous precipitation of the plant rhizosphere. This is achieved either by surface run off prevention,

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erosion or leaching [27]. It is applicable in the stabilization of metals in contaminated soil, sediment or water environments, which ensures they are not transferred to the food chain from the soil by translocating to other parts of food crops or to the underground water. This is possible by sorption via the root, precipitation and subsequent metal reduction around the plant rhizosphere, for instance the toxic Cr^{6+} is converted to Cr^{3+}, which is less toxic [42, 43]. Variation exists as to how prone a metal is to phytostabilization and is subject to its chemical character. This is evidenced in a comparative study to evaluate metal accumulation capacity of two aquatic macrophytes *Phragmites australis* and *Typha domingensis*, where both are found to stabilize As and Hg but inefficient in the phytostabilization of other metals [44].

Although phytostabilization offer some advantages over other phytoremediation techniques, it is however limited to temporary measure to deal with pollutants contamination owing to the fact that metals are only inactivated and their movement restricted, but still remains in the contaminated environmental compartment [45]. It is useful in emergencies, since it can rapidly immobilize pollutants from soil, water or sediment. Equally important, it ensures that contaminants are not translocated to other plant tissues by trapping most of it in the plant root [46]. Considering the strategies employed in phytostabilization, plants that can appropriately fall under this mechanism is their ability to tolerate and immobilize metals and other contaminants, low translocation capacity from root to plant aerial parts and of course extensive and fibrous tap root system [7]. Among several studies that reported plants species with these characteristics [47–49] demonstrating the phytostabilization of Zn, Pb, Cu and Cd by different plants in soil and sediment polluted environments.

2.1.4. Phytotransformation/phytodegradation

Phytotransformation or phytodegradation is another technique of phytoremediation where contaminants and other nutrients are chemically modified through plant metabolism and render associated contaminants inactive in both plant root and shoot tissues [6]. Plant metabolic enzymes act on the surrounding contaminants, thereby transforming them to a less toxic form, plants rhizosphere microbes also aid in the transformation process of the compounds [50]. Although this mechanism is mostly against organic contaminants, inorganic compounds such as metals were also suggested, in which case a strategy akin to phytostabilization is employed to convert toxic metals to less toxic form [51]. However, this technique seem less efficient and reliable compared to others in that it requires longer period of time, strict soil characteristic such as depth and underground water availability and often require soil amendments.

2.1.5. Phytovolatilization

In phytovolatilization, contaminants are converted in to a volatile form and released to the air via plants leaves stomata [27, 34]. However, this mechanism merely transfers contaminants from one environmental compartment to another, which may somehow return back to the original source (soil) by precipitation and hence could be less popular to other phytoremediation techniques especially phytoextraction and phytofiltration [34, 52]. It is commonly employed when treating groups of highly volatile metals like Hg and As. Phytovolatilization of As involves the conversion of elemental As to selenoaminoacids, such as selenomethione, which is modified by methylation to a volatile and less toxic form, dimethylselenide [53].

3. Metal hyperaccumulator plants

Several plants species are known to tolerate high concentration of toxic metals. Tolerant species are best described as excluders, where metal uptake and translocation to different tissue parts are limited. While others that are capable of accumulating higher concentrations with improved translocation from the root to shoot part of the plant, thereby significantly reducing its availability in the soil, and they do so with no visible sign of toxicity effects. To date, heavy metals have no standard definition by recognized bodies in the area. Various researchers use different characteristics and levels in their description such as atomic mass and number, density, chemical character as well as their toxicity; however, there appears no connection between such properties [54]. According to Wang and Chen [55], three categories of heavy metals arising from both natural and artificial sources are of interest, these includes valuable metals e.g. Ag, Au, Pd, Pt, harmful metals e.g., As, Cu, Co, Cd, Cr, Hg, Ni, Pb, and radionuclides such as Am, Th, Ra and so on. The non-biodegradability and stable nature of heavy metals suggests increased exposure to living species including humans [54], periodic reviews of toxic metals effects are documented by many research groups [56–58].

When determining hyperaccumulators of toxic metal, the most important factor is the concentration of the metal ion threshold. Therefore, plants can be regarded as hyperaccumulators, when capable of accumulating toxic metals concentration to about 50 to 100 times more than non-hyperaccumulator plants [13, 59]. For instance, the threshold for Zn and Mn hyperaccumulation in plant shoot is pegged at 1% of dry biomass, 0.01% for Cd and 0.1% respectively for Ti, Se, Sb, Pb, Ni, Cu, Cr, Co, and As [13, 60]. To date, few plant species are classified as hyperaccumulators, the majority of them (3/4) are tolerant to Ni and belongs to the Brassicaceae family native to Western Asia and Southern Europe, with up to 48 species implicated in Ni accumulation of around 3% dry shoot mass [60–62]. There is increasing interest in plant hyperaccumulators in recent times, owing to their potential use in metal contaminated soil and water detoxification [25, 63].

3.1. The role of metal chelators in hyperaccumulation

The phytoremediation of heavy metals involve many physiological, biochemical and molecular activities. In this process, especially phytoextraction involves the accumulation and translocation of heavy metals to plant tissues. Plant metal chelators or phytochelatins (PCs) and metallothioneins (MTs) are the most common transporter proteins for heavy metal phytoremediation. MTs are cysteine rich proteins that are famous for metal binding and greatly assist in the process of sequestration of metals in ionic form [64]. PCs are glutathione synthase products and they binds to heavy metals thereby forming central part of the phyto-detoxification mechanism [65, 66]. The induction of phytochelatins is induced by the activity of an enzyme, phytochelatins synthase, which is triggered by the activity of metal ions present [34, 67]. In an experiment to demonstrate the role of synthases, mutants in model plant *Arabidopsis thaliana* were shown to be hypersensitive to Hg and Cd, which is attributed to their inability to produce PCs [68]. On the other hand, MTs are genetically encoded metal binding peptides and usually bear low molecular weight. A number of studies demonstrate MTs role in the protection of plants against the toxicity of heavy metals in soil, sediment and water [65, 69, 70]. The expression of MTs and PCs, alongside organic acid synthesis, together functions in heavy metal uptake by plants and also their translocation to other tissue parts [42]. The expression of these natural chelators could be enhanced to increase the efficiency of heavy metal accumulation and translocation. Currently, there are many ongoing studies aimed at characterizing and identifying biomolecules involved in the transport and detoxification of heavy metals. This will aid in understanding the whole detoxification process involved in plants [28, 71], and to achieve this, the importance of comparative proteomic studies cannot be over-emphasized.

3.1.1. The shoot proteome

Plant shoot is an important tissue in phytoremediation process; it is especially responsible for accumulating the highest metals concentration when the subject plant employs phytoextraction technique, which is subject to the type of metal elements and bioavailability. In recent times, there has been an increased interest in the proteomics study of plant hyperaccumulators with the aim of characterizing and identifying proteins acting in metal sequestration and detoxification [72]. These are possible with the advancement in modern mass spectrometry techniques such as two-dimensional liquid chromatography matrix-assisted laser desorption/ionization time-of-flight (2D-LC/TOFMS), time of flight/mass spectrometry (TOF/MS), two-dimensional polyacrylamide gel electrophoresis (2D PAGE), and liquid chromatography- tandem mass spectrometry (LC–MS/MS). For instance, the proteome of many plants species including *Thlaspi caerulescens, Pteris vittata, Helianthus annuus* and *Agrostis tenuis* were recently searched for heavy metals detoxifying proteins; several key functional proteins were found that protect plants against oxidative stress, as well as those responsive to biotic and abiotic stress condition among others [12, 73, 74].

In the proteomics study of plant metal hyperaccumulators, comparison could be made, even when these studies are from different plants and metals. In 2005, [75] found that prolonged exposure of *Alyssum lesbiacum* to Ni in an optimized experimental condition induced only three proteins, and one of these proteins, iron superoxide dismutase (Fe-SOD), was demonstrated to have antioxidant activity [76], while the other two proteins were identified as chloroplast phosphoglycerate kinase and a transketolase both having a role in the carbohydrates metabolism. In *Anemone halleri*, photosynthetic protein (chlorophyll a/b binding) and membrane protein (photosystem II) were constantly translated and upregulated when treated with Zn and Cd, which is linked to the improved metabolic energy demand in this metal hyperaccumulating plant [77].

At high metal concentrations, increased proteins induction are involved in the defense against antioxidants and energy metabolism has been consistently observed; examples includes Renal Epithelial Protein (APX), Superoxide dismutase (SOD), cytochrome P450 and Glutathione S-transferase (GST). These suggest that, for the uptake, translocation and accumulation of heavy metals concentration on the shoot tissue, plants require the functional photosynthetic process as well as the activity of proteins that scavenge oxygen radical species [13]. Metabolic energy active proteins were also suggested to have important roles in metal tolerance by plants. The proteomes of *T. caerulescens* with variable tolerance to Cd and Zn metals were compared and there was a higher accumulation of the extrinsic subunit of photosystem II protein, which led to its stabilization in the more metal tolerant variant as against the less tolerant accession. In addition to GST and cytochrome P450 earlier mentioned, other proteins such as aspartate aminotransferase and thioredoxin are commonly found, and linked to the sequestration of xenobiotics including metals. GSTs have particularly been demonstrated to be up regulated in many other living species including bacteria and fungi treated with metals like Zn, Cu and Cd [78]; hence GST were suggested to confer resistance to toxic genes in these cells.

3.1.2. The root proteome

In plants, the root tissue is the first to be exposed to all potential toxicants whether in the soil or surface water and hence serve as the gateway route through which they can subsequently be translocated to other tissue parts. Plants diversity as to hyperaccumulators and non-hyperaccumulators exist, this is due to the fact that, while some species bear the complete mechanism of enhanced metal uptake and eventual translocation, others have limited sequestration capacity in their root vacuoles [79]. In non-hyperaccumulators plant roots, Zn transporters are only detectable in the absence of Zn, whereas in hyperaccumulators, there is constitutive expression of these proteins such as ZT1 even in Zn deficient condition [80, 81]. In *T. caerulescens*, the iron transporter protein IRT1 was found to be involved in Zn and Cd uptake [82], similarly, root proteome study of this hyperaccumulator and *A. lesbiacum* were conducted by Tuomainen, Tervahauta [83] to evaluate peptides involved in Zn and Cd hyperaccumulation. In these studies, various classes of proteins were identified, their availability and or abundance varies relative to metal exposure and accessions. As in the case with similar studies on shoot proteome of hyperaccumulators, ROS scavenging proteins were more abundant in the more metal tolerant accessions compared to the less metal tolerant species. It was concluded that the changes in the enzyme, superoxide dismutase (SOD) availability upon which Zn depends in the different accessions may be linked to ROS increase.

An important organelle, cell wall, in the plant root is also affected by its exposure to heavy metal stress. The putative protein, glycosyl hydrolase family 18, involved in the formation of cell wall structure was shown to be regulated in accordance to treatment conditions and accession. These proteins, which are particularly known to be involved in cell wall expansion, differ in terms of abundance between the root proteome of two accessions, which in turn also affect the capacity of metals uptake; higher Ni and Cd accumulation was observed in the variant with more protein abundance [13]. Despite the recent advancement in proteomics technology, root protein transporters are yet to be differentially identified. Indeed, this is in agreement with transcriptomics studies, with analyzed data suggesting the constitutive expression of metal genes transporters in plant metal hyperaccumulators [13, 81, 84].

3.2. The halophytes of Qatar are promising heavy metal hyperaccumulators

Some studies demonstrated the potential of several Qatari plants as good phytoremediation candidates, many among which are heavy metal hyperaccumulators. Examples includes species belonging to the genus *Zygophyllum*, which are as either metal tolerant or accumulators when tested on both polluted soil and wastewater media [85–89]. Others include Typha domingensis and *Phragmites australis* [2]. According to Carvalho and Martin [90], *Typha*

S/No	Plant species	Metal (s)	Metal accumulation (mg/kg)	References
1	*Atriplex halimus* subsp. *schweinfurthii*	Cadmium	606.51	[97]
	A. halimus L.	Cadmium	830	[98]
		Zinc	44	
2	*Arthrocnemum macrostachyum*	Lead	620	[99]
3	*Crucianella maritima*	Zinc	390	[99]
4	*Dittrichia viscosa*	Lead	270	[99]
5	*Tamarix smyrnensis* Bunge	Lead	800	[100]
		Cadmium	800	
6	*Typha domingensis*	Selenium	30	[90]
		Lead	59.13	[101]
7	*T. lotifolia* L	Cadmium	210	[102]
8	*Paspalum conjugatum* L. *Prosopis laevigata*	Lead	150	[103]

Table 1. Examples of phytoremediation studies using species belong to Qatari flora and/or their relatives.

domingensis remediated heavy metals from industrial waste water and solution cultures; similarly, members of the halophytes plant family Brassicaceae were reported to be important phytoremediation agents [3, 59, 91] and the tree plant *Prosopis juliflora* exhibited phytoremediation of heavy metals potential [92, 93]. Additionally, such as other plants, such as *Phragmites australis* that were previously shown to clean petroleum-polluted soils may be good candidates for the phytoremediation of typical oil and gas produced wastewater [94], others with similar potentials includes Medicago such as *Medicago sativa* and *Glycine max* which also demonstrated strong petroleum polluted soil phytoremediation activity [95, 96]. Some examples of other species tested for phytoremediation studies and their metal uptake capacity are summarized in **Table 1** above.

4. Conclusion

The accumulation of trace and heavy metals in the environment present a great risk to biota health. These contaminants are implicated in a wide range of human diseases and various long-term negative environmental consequences, thereby endangering overall sustainable development initiatives worldwide. Many conventional treatment strategies are widely practiced for the remediation of these contaminants. However, traditional remediation processes have many disadvantages, from complicating environmental pollution to high operational cost among others. Phytoremediation is one of the most promising alternatives in this regard, and laboratory experiments have demonstrated the capacity of hundreds of plants species to remediate different heavy metal contaminants. However, there still exist limitations in the application of this emerging technology. This may be linked to exposure to other stress factors in field conditions, and especially in extreme environments, which could significantly affect physiological function and general growth. An example is above the ground biomass accumulation, a key requirement for plants that is critical to phytoremediation success. The identification and selection of plants with higher metal uptake capacity or hyperaccumulators, even in the presence of other stress condition is therefore the objective of many phytoremediation studies in recent times. Additionally, our limited understanding on the molecular mechanism of heavy metal remediation, such as the exact role of transporter proteins is compounding progress in this area. However, it is obvious that several stress response molecules are key to the tolerance and or accumulation of heavy metal contaminants by potential phytoremediators. The halophytes are famous for their adaptation to stress environmental conditions, and hence could be the most suitable candidates in the search for appropriate heavy metal hyperaccumulators and consequent elucidation of mechanism of uptake. Indeed, these are significant steps essential to improving the efficiency of phytoremediation for large scale, field and industrial applications.

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Conflict of interest

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Author details

Kamal Usman, Mohammad A. Al-Ghouti and Mohammed H. Abu-Dieyeh*

*Address all correspondence to: dandelion@qu.edu.qa

Department of Biological and Environmental Sciences, College of Arts and Sciences, Qatar University, Doha, Qatar

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