Evaluation and Assessment of Metal(loids) Adsorptions by *Cenchrus ciliaris* L. in a Cement Contaminated Area

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The adsorbed amounts of Ni, Cu, Zn, and As metal(loids) were evaluated on *Cenchrus ciliaris* L. Results showed that *C. ciliaris* grass was able to collect these elements from soils in an active way. Several factors, such as the concentrations of elements in soils, pH, sunlight intensity, and temperature, contributed to enhance the adsorption of these toxic elements. The analysis for arsenic and phosphorus was conducted by Flameless Atomic Absorption Spectrometry and ICP-AES. It was found that the *C. ciliaris* plant managed to absorb phosphorus and keep the arsenic out of the root; thus, a selective behavior of absorption of elements by plants in contaminated sites was observed.

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INTRODUCTION

Cenchrus ciliaris L. is a grass native to the Middle East and western Asia (Humphreys 1967). It has an adaptation ability to thrive in several soil types. It grows in regions that receive small amounts of rain during the year, it establishes strong root systems, and it tolerates salt stress in soils (Marshall *et al.* 2012). This grass has been perceived as an appropriate source of animal food. It supports pastoral farming industries not only in Saudi Arabia but also in other countries because this plant is well suited for raising livestock (Grigg *et al.* 2000; Akiyama *et al.* 2005). Because of these characteristics, the plant faces the problem of contamination from environmental pollution. Vehicles emissions, factories releases, and oil industries might create a risk on the environment ending with plants accumulating toxicants. Then there can be an ongoing movement of these toxicants up to the top of the nutrition pyramid.

The *Cenchrus* plant is related to Poaceae (Image 1), which has specific traits that enhance growing in contaminated soils, for example shutting off the pumps of phosphate in the root cells upon arsenic contaminations (Meharg *et al.* 1994). However, some studies found that at high levels of contamination of arsenic, the roots become sinks of arsenic, while other trace metals such as cadmium and zinc continue to the shoot (Santos-Jallath *et al.* 2012).

One of the most contaminating sources to the environment is the cement industry. It has been listed by the Central Pollution Control Board (CPCB) as one of most polluting sources (Raajasubramanian *et al.* 2011). Cobalt, cadmium, nickel, lead, silicon, and chromium are pollutants that are released by cement factories and hazardous to the environment (Darweesh and Sayed 2014; Yadegarnia Naeini *et al.* 2019). Cement factories

products of heavy metals are able to travel for long distances from the source to affect grass and the availability of essential nutrients; those distances might reach 2000 meters (Soladoye *et al.* 2020).



Image 1. Cenchrus grass parts, panicle, stem, and leaves with height of approximately 21 cm

In a previous study by Al-Faifi and El-Shabasy (2021), *C. ciliaris* grown near a cement factory was anatomically studied. It was observed that the tissues of the plant were severely affected because of the cement dust falling on the regions in which the plants are grown. Therefore, in this study the accumulation levels of trace metals released from the cement factory into the plant tissues were evaluated. Nickel, copper, zinc, and arsenic levels in *C. ciliaris* roots and other green parts of the plant were assessed in the study. The nearest plants to the factory were also compared with plants grown far from the region of the cement industry.

EXPERIMENTAL

Site Description

The study was conducted near the cement dust factory in Jazan (operated first on 30th October 1981). The factory is located in Ahd-El-Masarha 70 km far southwest Jazan city, Kingdom of Saudi Arabia at 16°44'07.5"N latitude and 43°03'03.7"E longitude. The elevation of the land is approximately 132 m above sea level.

The land is mostly plain, with some randomly and unevenly distributed small hills. The factory was built there because the limestone rocky base is enough to establish a cement factory. Thus the cement klin dust is manufactured of a complex mixture of heavy metals including Cd, Ni, Zn, Cu, Fe, Mn, and Be.

The climate of the zone is affected by the location on the Red Sea shores; January temperature reaches 31 $^{\circ}$ C at maximum, while decreases to nearly 22 $^{\circ}$ C. In July the average temperature reaches nearly 33 $^{\circ}$ C.

Wind speed over Tihama is gentle mostly to moderate, and the velocities of it is from 38 to 109 km/h (21 to 59 knots). During the summer, the region is subjected to dust storms that are followed by heavy rains.

The relative humidity in January is 74%, and in August it is 66%. The average annual humidity is 68%, temperature is 30.4 °C, and rainfall is 139.7 mm (Dabbagh and Walid 1997; Masrahi 2014; Masrahi *et al.* 2017; Tounekti *et al.* 2018).

Sampling and Preparation

Both soil and plant samples were collected from the areas around the factory and from remote areas as a control in which no contamination is present. Six samples were collected; three were taken from the sites near the factory and three from the areas far from the factory. Samples near the factory were located approximately 400 m away from the cement factory. The control samples were taken from farms located outside the region of the factory.

Around nine bushes were gathered from each sampling area, and they were tagged as a sample from one site. The plants were immediately enclosed in nylon bags then transported to the lab for storing them in a refrigerator. The plant parts were cleaned and dried at room temperature at about 28 to 30 °C for one week. After that, shoots and roots were separated, then pulverized into a powder form (500 μ m) separately and weighed. Thus, the powder was the average of nine bushes collected from one site.

Soil samples were obtained as follows: Approximately 200 g samples were taken from each site at a depth of 10 cm to 20 cm, 0.5 kg per each, and mixed together. Soils were taken to the lab in nylon bags and kept in a cold container to prevent any biological reactions in it. Some soils were taken for physical analysis (Table 1). Soils were then subjected to acid washing followed by distilled water washing and stored in a plastic bag at room temperature for analysis. The soil pH was measured using a digital pH meter (with a pH reading meter (Model Jenway PHM 6) in (1:2.5), Reduction Potential (RP), soil to water ratio, electrical conductivity (EC), total dissolved salts (TDS), moisture content, water holding capacity (W.H.C.), soil texture, and organic matter were performed (Wilde *et al.* 1979).

Approximately 400 g of each of the whole powdered plant parts were soaked separately in 200 mL of distilled water and 95% ethanol for 72 h, in separate flasks. Extracts were obtained in an orbital shaker for 6 hours at room temperature. The extracts were washed free of ethanol and then concentrated using a rotatory evaporator at reduced temperature (40 °C) and pressure. The samples were filtered, and each residue was analyzed using an atomic absorption spectrometer Model: GBC932AA, Flameless Atomic Absorption Spectromer (Perkin Elmer 2380, USA) to measure the levels of inorganic elements: Ni, Cu, Zn, As, and P levels in the tissues (Sultana *et al.* 2009). Three replicates were obtained for each sample.

Next steps for the analysis of plant tissues and soils were initiated by ICP-AES (METHOD 3050B), where two grams of each sample was digested in nitric acid (2.5 mL conc. HNO₃) and hydrochloric acid (10 mL conc. HCL) to analyze for arsenic in soils and plant tissues. However, the plants and soils collected different amounts of adsorbate, where each sample of both soils and plants were taken from specific distances from the factory (Table 8). As phosphorus absorption has a relationship with the absorption of arsenic, it was analyzed in plants tissues. The available P in soils was evaluated using the Bray No. 1 Method (Irving and McLaughlin 1990). Three replicates per each sample of soils and plants. Three replicates for the soil and for the plants were taken from each point.

(1)

Accumulation Concentration

Heavy metal concentrations in soils and plants were calculated on the basis of dry weight. The Accumulation Concentration (AC), an index of the ability of the plant to accumulate a particular metal with respect to its concentration in soil substrate was calculated as follows,

$$BF = C_{plant} / C_{soil}$$

where C_{plant} and C_{soil} represent the heavy metal concentration in the plant and soil, respectively (Kumar and Fulekar 2018).

Translocation Factor

The translocation factor (TF) was calculated to define the relative translocation of metals from soil to root and leaf of the plant. TF is the ration of metal concentration in the shoots to the roots ([Metal] Shoot/[Metal] Root (Pankaj and Madhusudan 2018).

Statistical analysis

The coefficient of correlation, coefficient of determination, significance test value, standard error slope value, and F-test were used to test the soil physical properties (AS content) versus the soil chemical property (As contents) (Shaban 2005; Tamhane 2009; Welham *et al.* 2014; Ter Braak and Šmilauer 2002). The representation of statistical data was done by the linear regression approaches, which explored the extent effect for both soil variables (Maindonald 1992; Miller and Franklin 2002). P-values for significance tests of soil physical properties based on degrees of freedom were determined according to Dutilleul's (1993) approach.

RESULTS

The soil distances were classified into three different soil categories; low distance (10 to 100 m), medium distance (200 to 400 m) and high distance (500 to 700 m). Soil texture includes all particle sizes but soil low distance has the highest soil particles (4000 μ m) where soil high distance has the lowest soil particles (125 μ m >) (Table 1).

Soil Texture	4000 µm	2000 µm	500 µm	250 µm	125 µm	125 µm >
low distance	28.6±2.922	13.467±0.943	18.667±0.865	8.167±0.544	12.667±2.735	17.467±3.04
(10-100 11)						
medium	2.867±0.858	10.533±2.469	28.433±1.19	9.533±0.826	10.333±0.492	35.2±1.687
distance						
(200-400 m)						
high	6.007±1.761	14.067±0.818	23.133±0.634	15±2.765	18.5±0.698	3.5±0.294
distance						
(500-700 m)						

Table 1. Soil Texture in Three Different Soil Categories

The TDS gradually decreased with distance from the factory. This caused other soil physical parameters also to be decreased, such as pH, EC, and RP. On the other hand, TDS shortage causes organic matter and W.H.C. to increase (Table 2).

Table 2. Soil Physical Characterizations in Three Different Soil Categorie

	pН	Organic	TDS	EC	RP	W.H.C.
		matter (%)	(ppm)			
low distance	6.177±0.932	4.25±0.829	322±1.633	123.7±4.10	22.433±3.565	18±1.01
(10-100 m)						
medium	5.765±1.035	7±1	92.1±0.09	4.93±3.50	13.467±0.736	45±0.85
distance						
(200-400 m)						
high	5.863±0.666	8.667±3.399	4.65±0.05	0.667±0.125	10.767±0.45	31.5±13.5
distance						
(500-700 m)						

According to soil atomic absorption analysis, there are heavy metals other than P and As with different concentrations. Zn has the highest concentration while Ni and Cu have the lowest (Table 3).

Table 3. Elements Concentrations (ppm) Soils Near Factory by Atomic Absorption

Element	Р	Zn	Ni	Cu	As
Ppm	0.45	2.13	0.005	0.005	0.009

Table 4. Bioconcentration	Concentrations in Roots b	y Atomic Absorption
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Element	Near Factory (ppm)	Far from Factory (ppm)
Р	3.13	0.89
Zn	12.03	8.97
Ni	0.154	0.06
Cu	0.012	0.008
As	0	0

Table 5. Element Concentrations in Stems with Leaves in ppm by Atomic

 Absorption

Element	Near Factory (ppm)	Far From Factory (ppm)
Р	10.27	4.91
Zn	4.90	2.70
Ni	0.303	0.278
Cu	0.054	0.033
As	0	0

Finally, the accumulation and the translocation factors were calculated according to Ghoneim *et al.* (2014). All elements analyzed in this study exceeded one for the accumulation factor, except for the As, in both plant parts. A comparison between roots and stems found that the level of Cu was five times than that in roots, Ni in stems were double of that in roots and approximately the level of P in stems was four times the levels of it in roots (Table 6, 7)

Table 6. Accumulation Concentration for Each of Roots and Stems with all

 Elements

Element	Accumulation Concentration		
	Roots (ppm)	Stems (ppm)	
Р	6.96	22.82	
Zn	5.65	2.30	
Ni	30.80	60.60	
Cu	2.40	10.80	
As	0.00	0.00	

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Element	Near Factory (ppm)	Far From Factory (ppm)
Р	3.28	5.52
Zn	0.41	0.30
Ni	1.97	4.63
Cu	4.50	4.13
As	0.00	0.00

In P studies using ICP-AES, the soil and plant samples were taken in interval distances from the factory; 10, 50, 100, 200, 300, 400, 500, 600 and 700 m. The P concentration in the soil was more or less the same, while it was increased in plant stems in high distance as shown in (Table 8) (Fig. 1).

Table 8. Phosphorus Concentratio	ns (ppm) in Both	Stem and Soil by ICP-AES
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Distance (m)	Stem (ppm)	Soil (ppm)	AC
10	21.35	0.36	59.31
50	18.99	0.45	42.20
100	10.2	0.37	27.57
200	13.84	1.25	11.07
300	10.63	1.25	8.50
400	47.78	1.25	38.22
500	33.31	1.50	22.21
600	6.8	1.50	4.53
700	42.68	1.50	28.45



Fig. 1. Phosphorus concentrations (ppm) in both stem and soil

In the study by ICP-AES, the As- soil concentrations were decreased gradually, while the plant concentrations were more or less constant except at 500 m for root concentration (Table 9) (Fig. 2).

Distance (m)	Root (ppm)	Stem (ppm)	Soil (ppm)
10	0.07	0.09	0.23
50	0.09	0.05	0.23
100	0.07	0.04	0.23
200	0.07	0.05	0.17
300	0.07	0.04	0.17
400	0.07	0.04	0.17
500	0.2	0.05	0.13
600	0.06	0.04	0.03
700	0.07	0.04	0.01

Table 9. Arsenic Concentrations (ppm) in Root, Stem and Soil by ICP-AES



Fig. 2. Arsenic concentrations (ppm) in roots, stem and soil

Accumulation concentration was increased in the high distances while translocation factor is gradually decreased especially at 500 m (Table 10) (Fig. 3).

Table 10. Accumulation Concentration and Translocation Factor for As
Concentrations in the Plant

Distance (m)	AC	TF	
10	0.39	1.28	
50	0.22	0.56	
100	0.17	0.57	
200	0.29	0.71	
300	0.24	0.57	
400	0.24	0.57	
500	0.38	0.09	
600	1.18	0.67	
700	4.00	0.57	



Fig. 3. Accumulator Concentration and Translocation Factor for As Concentrations

Due to statistical analysis, the coefficient of correlation between soil physical properties and As concentration in soil showed negative values for moisture, organic matter, and W.H.C., while pH, EC, RP, and TDS showed positive ones. Simple linear regression (SLR) equations represented the previous data analysis as scattered plot graphs for exponential curves of studied comparative parameters (Table 11) (Fig. 4).

	Coefficient of correlation	Coefficient of determination	Significance test value	Standard error slope value	F-test	P-value
WHC	-0.344	11.809%	-0.366	145.221	>100	<0.0001
RP	0.89	79.205%	1.952	31.9	>100	<0.0005
EC	0.786	61.838	1.273	494.164	>100	<0.0001
TDS	0.91	82.895%	2.201	776.552	>100	<0.0001
Organic matter	-0.951	90.494%	-3.085	7.878	>100	0.0031
PH	0.601	36.116%	0.752	1.971	6.0782	0.2826
Moisture	-0.568	32.289%	-0.691	25.316	>100	0.0021

Table 11. Statistical Analysis Interaction between Soil Physical Parameters and

 As Concentrations in Soil

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Organic matter **b)** the relationship between As concentration in soil and the organic matter in soils

5.6

7.0

7.7

0.24

0.10

0.12

0.05

4.9

AS soil



c) the relationship between As concentration in soil e and reproduction potential



d) the relationship between As concentration in soil and total dissolved solids

Fig. 4. Simple linear regression of the significant relationships between soil physical parameters and as concentrations in soil

DISCUSSION

Nickel, Zinc, and Copper

The absorption of an element under the stress of its availability in the medium is facilitated (Dimkpa *et al.* 2008). It appears that all elements were well absorbed by the plant on the site of the study in an efficient way except for arsenic (Table 5).

Nickel, zinc, and copper are considered important elements to plants including grass, and the deficiency of them leads to several morphological and physiological defects (Pätsikkä *et al.* 2002; López *et al.* 2011; Zhao *et al.* 2011 and Awofolu *et al.* 2017). Thus, the contents in soils and the absorption facilitation might be reasons why this plant stored high levels of Ni, Zn, and Cu in the green parts.

It was found that high amounts of light support the absorption of Cu in plants such as in some algae (Küpper *et al.* 2002), and the *Cenchrus* grass in this study were grown on a subtropical area that receives enough irradiation.

Dinelli and Alessandra (1996) studied the absorption pattern of each of *Salk* spp. (Salicaceae), *Populus nigra* (Salicaceae), and *Silene armeria* (Caryophyllaceae) on contaminated sites with levels of Cu and Ni where the plants absorbed high contents of these elements at high levels during the cold months. The current study was in November, which is during a cold season. Each of Ni and Cu in this study were at high contents in the plant tissues compared to that of soils. Thus, the environmental temperature plays a role in boosting plants to absorb these two elements.

Ghoneim *et al.* (2014) discussed that *Cenchrus ciliaris* behaves as an accumulator more than hyperaccumulator. *Cenchrus ciliaris* L. is an indigenous plant to the region of Jazan in both shorelines and rocky areas, and it is reported that accumulator species are usually native to specific soils (Baker *et al.* 1989). In addition, this plant is an adaptive plant to contaminated soils (Akram *et al.* 2007).

According to McGrath and Zhao (2003), with bioconcentration factor greater than 1.0, the plant is considered as hyperaccumulator. Thus, *Cenchrus* plants could follow the hyperaccumulator behavior on the contaminated site of the study reported here (Table 6). Values of bioconcentrations reported for this study are more than one for all elements but arsenic.

Therefore, the area might be posing a risk to animals living there. For example, grazing should be banned in such areas. Not only are the livestock at risk, but even the biodiversity. Human life is at a great risk because cattle feed on the plants then the human feed on animals in area.

Arsenic

In aerobic conditions, arsenic is absorbed by root cells in the form of arsenate (As (V)), through the transporters of the inorganic phosphorus (Pi) in the roots. The arsenic is either detoxified by cellular partitioning and stored in the vacuole of the cell as arsenite (As (lll)) or exuded back to the soils as arsenate (Li *et al.* 2016). In addition, arsenic enters the root cells through silicon transporters, where it undergoes reduction and partitioning to mitigate its toxicity (Abbas *et al.* 2018) (Fig. 5).



Fig. 5. Simple diagram shows some known pathways of As from soil to the shoot.

Arsenic in soil was at average levels similar to or greater levels than those of Cu and Ni (Tables 3, 9). However, the concentrations in plant tissues is zero or unconsidered (Tables 3, 9). Therefore, the absorption is either genetically resisted by the plant or restrained in the medium, or both conditions might take place. One hypothesis is that the uptake of As is inhibited from the soils. In other words, As is adsorbed to the solid phase of the soil tightly and its availability to plants is affected. The adsorption of both As and P is competitive in soil (Roy *et al.* 1986; Gao and Mucc 2001; Strawn 2018), where either one replaces the other in soil minerals. The author hypothesizes that as the levels of P in soil increase, the competition between As and P in soil minerals increases, releasing As into the soil solution making it available to plants and *vice versa.* After a survey, Gunes *et al.* (2009) supporting this hypothesis. The competing P content in the studied soils might be low and not enough to cause a release of As from soil minerals to the soil solution. Thus, As absorption was observed to be at a low rate.

Plants develop a strong root system to extract elements from soil minerals when their contents are low in soil solution (Abbaspour *et al.* 2012). These root systems were observed in *Cenchrus* harvesting P even if it was low in soils (Tables 4, 5, and 8). As a result, these roots system might reach As in soil minerals, and as it is an analogue to P ((Tu and Ma 2003), roots should take up As no matter what.

Meharg *et al.* (1994) and Remy *et al.* (2012) found that the grasses *Holcus lanatus* L. and *Arabidopsis thaliana* are able to suppress the uptake of arsenate by shutting off the uptake system of phosphate on contaminated sites and upon the plant starvation for P. Precisely, As may cause enzymatic disruption then the phosphate flow stop (Kabata-Pendias and Mukherjee 2007). Several studies have reported that members from Poaceae are resistant to As such as (Rocovich and West 1975; Pollard 1980; Macnair and Cumbes 1987). *Cenchrus* is a member of Poaceae, thus it might be resistant to As uptake too. Nonetheless, it is preferable here to say that when the As content is low in soils because Santos-Jallath *et al.* (2012) found that at high pressure of As (183 to 14,660 mg/kg) in soils, roots of *Cenchrus* store high As. However, the shoot in their study did not store As, which

might strengthen the hypothesis that *Cenchrus* does not absorb As and the storage of it in the root in their study was due to a mechanical break of As through the root tissues.

The ratio of plant/soil P might be related to the preferential behavior of the plant to take up P even when As is present in soils (Tables 6, 7, 8, and 9). This behavior of the plant with P and As should be further studied and investigated. The reasoning for why plants manage to take up the essential element P compared to the non-essential element As needs further study. At the points of 600 and 700 meters, the bioconcentration factor increased, and that might be related to some physiological aspects of the plants, whereas the distance of the plants from the source of contamination increases the defense of the plant not to absorb arsenic decreases. It has been hypothesized that as arsenic concentration decreases in soil, its plant uptake increases (Rajkumar *et al.* 2009).

If the plant develops a strategy that enables it to avoid As in the soil, the analogy of As-P might not be always the explanation why plants take up As concurrently with P when both are present in soils. Therefore, based on the results of this study, it is hypothesized that the plants smartly adapt when in contaminated soils not only to tolerate the contamination upon absorption and accumulation, but also to select the element that is necessary to absorb. Elias *et al.* (2012) found that in some bacteria, the proteins that bind phosphate were able to differentiate between phosphate and arsenate in an environment rich with arsenate. Thus, *Cenchrus* might have an equivalent discrimination behavior in the presence of both As and P.

Therefore, both factors, a genetic factor and the soil conditions of As–P contents, might play a crucial rule here in restraining As availability and absorption by *Cenchrus ciliaris*. In addition, saline soils also cause a reduction in As accumulation in plant (Zhou 2019). The soils in the contaminated sites were relatively saline. Nonetheless, the reduction might leave some considerable detected As in the plant tissues, but none was at the interest present in the plant used in this study.

Halophytes are known to be able to dismiss the toxic metal(loids) from their salt regulating organs; *Atriplex halimus* L. is one of several halophytes that are able to regulate Pb and Cd elements with salts regulation using salt glands (Manousaki and Nicolas 2011). Thus, *Cenchrus* spp. is a halophyte (Hall 2000; Siddiqui *et al.* 2016) and might reduce As by excretion through the same organs. However, a value of zero or strongly minimized stems values to those of soils for As contents for both methods used in this study to maximized values of the other toxic elements suggest that this plant has no As absorption.

Therefore, *C. ciliaris* could be a plant that has a selective absorption. While P is absorbed, As is not. More studies with a higher number of samples are needed for the future investigations on As and the *Cenchrus* plant.

It would be more beneficial to gene-scan this plant concurrent with more intensive field studies. Thus, if there is a gene that renders selection trait to the plant, it might be opening a new bioengineering future to save more plants and even crops that can avoid contamination on arsenic-contaminated sites. The difference in uptake values of both P and As is regarded to the importance of phosphorus to the plant. AC values exceed TF ones, which indicates that there is a defensive mechanism for more As uptake due to its plant retention by sorption and precipitation. This mechanism defense is translated as redox arsenic condition, which changes the toxicity of arsenic to form into non-toxic organic forms (Tangahu *et al.* 2011). P values reflects that pH is the most significant character that is affected due to the alteration of chemical composition in the soil. The negative values of coefficient of correlation confirm that there are bad effects on soil physical properties.

Finally, all statistical analysis confirms that arsenic plant condition is the brilliant excellent example of heavy metal manipulation and assessment versus the plant response adaption.

CONCLUSIONS

Poaceae has specific absorption behavior in contaminated soils, where it shuts off the P pumps in roots in case of contamination with As in soils. This protects the plant from the toxicity. *Cenchrus ciliaris* has shown that it can select the essential element that is necessary for the biological activities and growth.

- 1. The *Cenchrus* plant managed to absorb P and keep the As out of the root. Thus, a selective behavior of absorption might be developed in this plant.
- 2. The *Cenchrus* plant shows a high metal uptake ability. Metals, such as Ni, Cu, and Zn, have been efficiently accumulated by the plant and well extracted from the soils. Thus, this plant can be used for phytoextraction of toxicants from soils and cleaning up the soils.
- 3. In addition, *Cenchrus* can be used for photo stabilization. Soils near the contamination sources should be stabilized and not allowed to move to further areas. This plant and its root system were able to partially prevent the contaminants from migrating to other areas.
- 4. The amount of light and temperature, soil moisture, root system of the plant, and the ongoing emitting of contaminants to the soils near the factory are factors that cause the contamination in the plants surrounding the area.

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