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Potentiality of *Amaranthus viridis* (L.) to accumulation of heavy metals and its relation to protein profile

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Abstract

The present study was undertaken to assess the levels of heavy metals (Cd, Fe, Ni and Zn) in soil and plant parts. The phytoremediation potential of *Amaranthus viridis* (amaranth) was determined by calculating the bioconcentration factor (BCF) and translocation factor (TF). Soil and plant samples were collected from eight different sites in their native habitats, distributed along Ismailia irrigation canal located in the east of the Nile Delta region, Egypt. Roots of amaranth had the higher concentrations of Cd, Fe and Ni means which were 2.75, 547.3 and 7.25 mg g^{-1} DW at sites SH8, SH6 and SH4 respectively, as compared to the other sampling sites. Whereas, shoots had the higher concentrations of Zn (283.6 mg g^{-1} DW) at site SH5. All sites were found with BCF more than 1 for all heavy metals. Except for Zn, TF values of Cd, Fe and Ni at all sites were lower than one. Depending on BCF and TF results, amaranth showed significant potential for phytostabilization of Cd, Fe and Ni and phytoextraction of Zn as well. Moreover, the protein profile showed different bands with varied molecular weights. Both relationships between studied accessions and similarity were elucidated, with high similarity (96% between SH2 and SH3 accessions), lowest similarity (72% between SH4 and SH8) and degree of polymorphism calculated as 62.5%. The variation in the similarity index may be due to the induction of a variety of proteins under heavy metal stress and also ecological features in various sites that cause protein polymorphism.

Keywords

Amaranth; heavy metals; phytoextraction; phytostabilization; protein bands

Introduction

The public health is seriously threatened by heavy metals, and their negative impacts are also reflected on plants and animals. Therefore, studies on the effects of heavy metals have gained more attention for the health of the habitats and their inhabitants (1). The negative impacts of excessive heavy metals on environment appear in water and soil pollution and their phytotoxicity, all of these have substantial health concerns (2). Additionally, heavy metals cause a noticeable decrease in crop yield worldwide (3). Plants record several responses to heavy metals stress, appear at many levels such as molecular, cellular, morphological and the whole plant (4, 5).

Phytoremediation become a popular method for remediation plans in developing communities due to the ease of application and low cost. Native species used in phytoremediation strategy to remove contaminants from soil and water (6). Phytoremediation of heavy metals using the annual herb *Amaranthus viridis* (L.) that belongs to family Amaranthaceae with common name wild amaranth was investigated by many studies (7-9). These studies determined the different heavy metals (Cd, Fe, Ni and Zn) and assessed the phytoremediation potentiality of

amaranth. *Amaranthus viridis* is naturally growing in polluted soil sites, which were chosen to represent different sources of pollution in Egypt. The primary sources of heavy metals include the manufacturing activities, development techniques, wastewater irrigation systems, and agricultural practices (10). The metal limitations for plants to be regarded as hyperaccumulators are 1 mg g^{-1} for Cd, 10 mg g^{-1} for Ni, and 100 mg g^{-1} for Zn (11). The phytoremediation potentiality of a plant can be estimated by calculating values of the bioconcentration factor (the ratio of the metal concentration in the plant to that in soil) and translocation factor (metal concentration ratio in plant shoots to roots) (12). A plant species may have the ability to remove metals through phytoextraction if both the BCF and TF values are more than one, whereas the TF value lower than one indicates that the plant may be able to phytostabilize metals in roots (13).

Many biochemical markers were created to supplement morphological traits in plant diversity analysis and for identification and characterization of genotypes (14, 15). Various studies have shown that sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) can be used to assess the difference and similarity between plants (16). Numerous studies on plant proteomics have shown that different classes of proteins are produced in plants growing in varied polluted soils, where they are essential for cell defense or tolerance to heavy metals. Chaperones and proteins abundances involved in signaling routes or detoxification mechanisms are expanded (17). Protein electrophoresis was successfully applied in order to assess the likely mutagenesis potential created by the different accumulated contaminants (18).

This study aimed to determine the concentration levels of heavy metals (Cd, Fe, Ni and Zn) in soil and their transfer and accumulation in amaranth, roots and shoots. Subsequently, investigation of the amaranth phytoremediation potential of heavy metals was conducted by calculating the bioconcentration factor (BCF) and the translocation factor (TF) and estimating the correlation between heavy metal accumulation and the protein profile using SDS-PAGE based on polymorphism between the studied samples.

Materials & methods

Plant and soil collection

Amaranthus viridis and soil samples were collected from their natural habitats in the study area. The study area includes eight contaminated and industrial sites distributed along Ismailia irrigation canal located in the east of the Nile Delta region, Egypt. Code and GPS record of these sites shown in (Table 1).

Table 1. Code and GPS record of the 8 selected sites.

Selected sites	Code	GPS record
Ismailia Canal Intake from Nile	SH1	30° 06' 24.48" N 31° 14' 54.58" E
Seriakos Water Treatment Plant	SH2	30° 11' 0.08" N 31° 18' 45.54" E
El-Nasr Industrial Charcoal Company	SH3	30° 21' 27.62" N 31° 27' 14.92" E
Oil and Detergents Nile Company	SH4	30° 07' 30.40" N 31° 17' 13.20" E
Lion Glue Factory	SH5	30° 06' 33.40" N 31° 15' 23.13" E
Petroleum Pipelines Company	SH6	30° 08' 54.51" N 31° 17' 46.50" E
Swailem Vitrified Reformed Clay Factory	SH7	30° 11' 47.73" N 31° 19' 07.16" E
Abou-Zabal Chemical Fertilizers Factory	SH8	30° 16' 25.44" N 31° 22' 40.04" E

Plant analysis

Firstly, three amaranth samples were collected from each site, rinsed with distilled water then separated into root and shoot and digested in acids mixture, concentrated nitric acid (69%) and perchloric acid (60%) in the ratio of 4:1. Cd, Fe, Ni and Zn were determined in all samples by atomic absorption and flame photometer Shimadzu Model AA640F (Japan) and expressed as mg g^{-1} dry weight (19). Growth parameters of the plant collected from each site were recorded such as root and shoot lengths (cm), fresh weights of root and shoot (g).

Soil analysis

Three replicates of soil samples were taken from each site, then air dried and sieved in 2 mm mesh size at laboratory. Cd, Fe, Ni and Zn were estimated in oven dried soil. Levels of heavy metals determined as mg g^{-1} dry weight (19). The studied heavy metals were chosen according to previous study carried out by Yap et al (7). The concentrations of these heavy metals in soil samples collected from the study sites exceed the standard levels of WHO/FAO (20).

Phytoremediation potential

Phytoremediation capacity of amaranth was determined by two indices, bioconcentration factor (BCF) and translocation factor (TF). The BCF was calculated by dividing the concentration of heavy metal in a plant by its concentration in the soil. While, TF is the concentration of an element in shoots by the concentration of the same element in roots (21).

BCF = Metal concentration in plant / Concentration of the same metal in soil

TF = Shoot metal concentration / Root metal concentration

SDS-PAGE Analysis

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was used to distinguish the protein pattern in *A. viridis* shoots from all eight locations (22). At 4 °C, 0.5 g of fresh shoot was macerated in 1 ml of extraction buffer (62 ml Tris-HCl, pH 6.8, 20 ml 10 % glycerol, 40 ml 2% SDS) with a mortar and pestle. After centrifuging the extracts at 12,000 rpm for ten minutes, the supernatant was put to a fresh tube. It was subsequently stored in a deep freezer at -20°C until electrophoretic analysis was

performed. A volume of 30 µl of protein extract was mixed with an equal volume of sample buffer " 20 ml extraction buffer, 10 µl mercaptoetanol and 10 µl bromophenol blue", the mix was vortexed for 20 second and about 25 µl of this mixture were loaded on the gel. Total extracted protein was analyzed by denatured polyacrylamide gel electrophoresis. The electrophoresis framework was programmed to 60 V for 30 min (stacking gel). Then, the resolving gel can be run at 120 V for 2 hours at room temperature. Protein gels were stained for 2 h with 0.25% Coomassie brilliant blue R 250 stain and then destained for 2 h (200 ml ethanol, 57 ml glacial acetic acid and 1 L distilled water). The protein banding profile in the gel was photographed while the gel was wet. The number of bands for each population was scored and the percentage of polymorphic bands was determined. Each band was considered a single locus and scored as one for presence and zero for absence.

Statistical analysis

One-way ANOVA was applied to analyze data depending on means and standard error (SE) values. The p-value < 0.05 was considered statistically significant using SPSS program version 23 and followed by Duncan Multiple Range Test (DMRT) for comparisons between means. The molecular data were analyzed using the PAST-pc 4.2 software program then the clustering, principal component analysis and similarity coefficient were determined.

Results

Growth characteristics of Amaranthus viridis

The highest growth parameters of amaranth was recorded at site SH8 whereas, SH1 had the lowest values. The highest root and shoot lengths were 35.4 ± 0.18 and 76.4 ± 0.33 cm at site SH8 and the lowest values were 14.2 ± 0.15 and 30.64 ± 0.36 cm at site SH1 (Fig. 1). The highest values of root and shoot fresh weight were 4.13 ± 0.07 and 17.23 ± 0.13 g in site SH8 and the lowest values (0.65 ± 0.03 and 6.02 ± 0.1 g) were in site SH1 (Fig. 2).

Levels of heavy metals in soil and plant parts

Significant variations in heavy metal levels among the soils that were collected from different sites (Table 2). Cadmium concentration in the soils ranged from 0.144-0.777 mg g^{-1} DW. Soil Cd concentrations recorded significant variations among SH4, SH5, SH6, SH7 and SH8 sites. While Fe concentrations in soil ranged from 0.42-1.83 mg g^{-1} DW. The concentration of Fe values showed significant variation among the all soils of study sites except SH1 and SH3. With regard to Ni and Zn, their concentrations ranged from 0.23-2.26 and 1.49-6.74 mg g^{-1} DW, respectively and significant variations were detected among the all soil sites.

Table (3) showed different concentrations of heavy metals in plant parts, roots and shoots. Sites SH7 and SH8 had the highest levels of Cd in roots and shoots of wild amaranth when compared with other sites. Cadmium concentrations in amaranth roots and shoots were 2.75 and 2.10 mg g^{-1} DW at SH8 respectively. The analyzed data

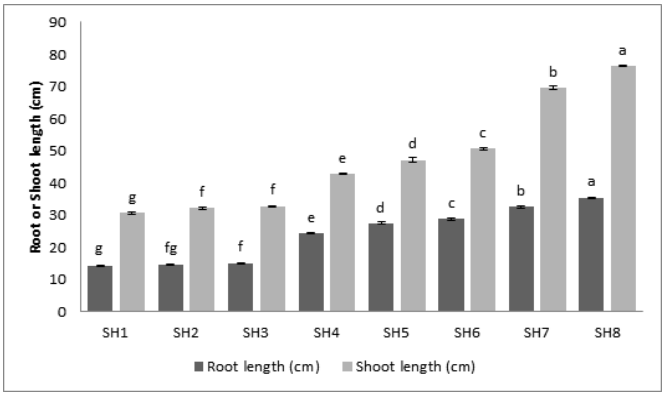


Fig. 1. Root and shoot length of amaranth at the 8 sites. The means followed by the same letter are not significantly different at P < 0.05.

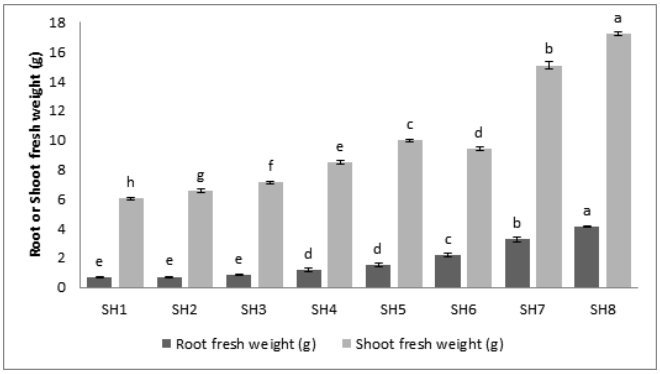


Fig. 2. Root and shoot fresh weight of amaranth at the 8 sites. The means followed by the same letter are not significantly different at P < 0.05.

Table 2. Heavy Metal concentrations of soils collected from eight sites.

	Heavy Metal concentrations (mg g-^{1}DW)			
Sites	**Cd**	**Fe**	**Ni**	**Zn**
SH 1	0.144 ^{f} ± 0	0.420 ^{g} ± 0.02	0.233 ^{g} ± 0.01	1.490 ^{f} ± 0.15
SH 2	0.157 ^{f} ± 0.01	0.557 ^{f} ± 0.01	0.337 ^{fg} ± 0.03	2.080 ^{e} ± 0.05
SH 3	0.171 ^{f} ± 0.01	0.447 ^{g} ± 0.01	0.433 ^{ef} ± 0.01	2.330 ^{e} ± 0.1
SH 4	0.453 ^{c} ± 0.02	1.120 ^{e} ± 0.06	2.267 ^{a} ± 0.13	3.193 ^{d} ± 0.1
SH 5	0.230 ^{e} ± 0.02	1.547 ^{b} ± 0.01	0.570 ^{de} ± 0.03	6.747 ^{a} ± 0.13
SH 6	0.317 ^{d} ± 0.01	1.830 ^{a} ± 0.01	1.580 ^{b} ± 0.04	3.393 ^{d} ± 0.07
SH 7	0.733 ^{b} ± 0.02	1.243 ^{d} ± 0.02	0.710 ^{d} ± 0.02	4.150 ^{c} ± 0.09
SH 8	0.777 ^{a} ± 0.01	1.347 ^{c} ± 0.02	1.127 ^{c} ± 0.07	5.513 ^{b} ± 0.06
F	399.44	406.55	153.57	323.05
P value	***	***	***	***

Notes: The columns with the same letter are not significantly different at P<0.05. The *** symbol next to each p-value in the table indicates that the differences between the sites are highly significant at p < 0.001.

Table 3. Heavy metal concentrations (mg g^{-1} DW) in roots and shoots of amaranth at the eight sites.

Sites	Cd		Fe		Ni		Zn	
	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots
SH 1	0.743 ^{g} ± 0.01	0.253 ^{e} ± 0.01	159.00 ^{h} ± 1.53	34.33 ^{h} ± 2.33	1.00 ^{g} ± 0.07	0.553 ^{f} ± 0.03	74.00 ^{f} ± 2.31	82.33 ^{f} ± 1.2
SH 2	0.753 ^{g} ± 0.01	0.250 ^{e} ± 0.02	178.33 ^{g} ± 1.2	40.33 ^{g} ± 1.45	1.14 ^{g} ± 0.04	0.657 ^{f} ± 0.02	74.00 ^{f} ± 2.08	83.00 ^{f} ± 1.53
SH 3	0.857 ^{f} ± 0.01	0.340 ^{e} ± 0.02	202.00 ^{f} ± 1.53	45.33 ^{f} ± 0.88	1.62 ^{f} ± 0.07	0.737 ^{f} ± 0.01	81.33 ^{f} ± 0.88	84.67 ^{f} ± 0.88
SH 4	1.727 ^{c} ± 0.01	1.033 ^{c} ± 0.09	349.66 ^{e} ± 3.28	62.00 ^{e} ± 2.52	7.25 ^{a} ± 0.13	3.257 ^{a} ± 0.13	175.66 ^{d} ± 1.76	180.33 ^{d} ± 2.03
SH 5	1.107 ^{e} ± 0.05	0.517 ^{d} ± 0.01	365.66 ^{d} ± 2.96	73.00 ^{d} ± 1.73	6.33 ^{b} ± 0.14	2.150 ^{b} ± 0.09	283.66 ^{a} ± 3.48	301.00 ^{a} ± 2.08
SH 6	1.447 ^{d} ± 0.02	0.633 ^{d} ± 0.01	547.33 ^{a} ± 2.19	157.66 ^{a} ± 1.2	3.49 ^{e} ± 0.2	0.940 ^{e} ± 0.02	151.66 ^{e} ± 3.28	157.66 ^{e} ± 1.86
SH 7	2.357 ^{b} ± 0.02	1.727 ^{b} ± 0.04	403.66 ^{c} ± 2.03	110.00 ^{c} ± 1.15	4.21 ^{d} ± 0.11	1.340 ^{d} ± 0.05	195.33 ^{c} ± 2.91	226.00 ^{c} ± 3.79
SH 8	2.757 ^{a} ± 0.01	2.100 ^{a} ± 0.06	480.00 ^{b} ± 2.31	140.33 ^{b} ± 0.88	5.14 ^{c} ± 0.07	1.787 ^{c} ± 0.02	237.00 ^{b} ± 2.08	250.66 ^{b} ± 2.91
F	1033.14	272.20	18903.84	847.62	444.92	246.70	1185.94	1249.54
p value	***	***	***	***	***	***	***	***

Notes: The columns with the same letter are not significantly different at P<0.05. The *** symbol next to each p-value in the table indicates that the differences between the sites are highly significant at p < 0.001.

showed that Cd concentrations in root and shoot were significant among the study sites except SH1 and SH2. Roots and shoots of amaranth at SH6 contained the highest concentration of Fe (547.33 and 157.66 mg g^{-1} DW respectively). The concentrations of Fe in root and shoot were significant among the all sites. While the highest levels of Ni in roots and shoots were 7.25 and 3.25 mg g^{-1} DW respectively, recorded at SH4 site. Ni concentration in root was significant in all sites except SH1 and SH2 but in shoot were non-significant in SH1, SH2 and SH3. On the other hand, site SH5 showed the highest levels of Zn when compared with other sites. The maximum Zn content of amaranth roots and shoots (283.66 and 301.00 mg g^{-1} DW). Zn concentrations in root and shoot were significant among all sites except SH1, SH2 and SH3.

Roots had higher concentrations of Cd, Fe and Ni than shoots and soils at all study sites. While Zn recorded its highest concentration in shoots than roots and soils at all study sites.

Bioconcentration and translocation factors (BCF and TF) of heavy metals

Phytoremediation capacity of amaranth was calculated using the bioconcentration factor (BCF) and translocation factor (TF) as shown in (Table 4). BCF and TF values ranged from 6.12-7.10 and 0.331-0.762 for Cd, 283.55-555.24 and 0.178-0.292 for Fe, 5.42-7.83 and 0.27-0.57 for Ni and 71.53-111.68 and 0.946-1.94 respectively for Zn respectively. The present data showed that the BCF of Cd, F and Ni by amaranth roots at all sites were more than one but the TF is less than the unit. In contrast, BCF of Zn is less than one while the TF is more than the unit in all study sites (Table 4).

SDS-PAGE analysis and protein banding patterns

The electrophoresis analysis of protein extract using discontinuous SDS-PAGE gel for the eight amaranth accessions was shown in Figure (3). The dendrogram based on protein bands resulted from SDS-PAGE analysis for the eight amaranth accessions was divided into two subclusters at a total distance of 2.2. The first subcluster was divided into two groups: the first group included SH1, SH2 and SH3

Table 4. Biconcentration and translocation factors of amaranth at the eight sites.

Sites	Cd (mg g^{-1})		Fe (mg g^{-1})		Ni (mg g^{-1})		Zn (mg g^{-1})	
	BCF	TF	BCF	TF	BCF	TF	BCF	TF
SH 1	6.914 ^{ab} ± 0.23	0.341 ^{d} ± 0.01	461.696 ^{d} ± 19.87	0.216 ^{a} ± 0.01	6.670 ^{b} ± 0.16	0.560 ^{a} ± 0.06	106.898 ^{a} ± 9.59	1.114 ^{a} ± 0.03
SH 2	6.417 ^{abc} ± 0.2	0.331 ^{d} ± 0.02	393.434 ^{e} ± 11.88	0.226 ^{a} ± 0.01	5.429 ^{cd} ± 0.47	0.573 ^{a} ± 0.01	75.556 ^{de} ± 2.24	1.122 ^{a} ± 0.02
SH 3	7.003 ^{ab} ± 0.11	0.396 ^{cd} ± 0.01	555.240 ^{c} ± 23.36	0.224 ^{a} ± 0	5.455 ^{cd} ± 0.04	0.454 ^{b} ± 0.02	71.538 ^{e} ± 3.38	1.042 ^{b} ± 0.02
SH 4	6.127 ^{cd} ± 0.47	0.598 ^{b} ± 0.05	370.051 ^{e} ± 23.16	0.178 ^{b} ± 0.01	4.675 ^{d} ± 0.31	0.450 ^{b} ± 0.03	111.686 ^{a} ± 3.34	1.027 ^{b} ± 0.02
SH 5	7.100 ^{a} ± 0.29	0.469 ^{c} ± 0.02	283.550 ^{e} ± 2.53	0.199 ^{c} ± 0	5.379 ^{cd} ± 0.26	0.340 ^{c} ± 0.01	86.739 ^{cd} ± 2.16	1.943 ^{c} ± 0.01
SH 6	6.574 ^{abc} ± 0.1	0.438 ^{c} ± 0	385.240 ^{b} ± 3.33	0.288 ^{bc} ± 0	7.771 ^{a} ± 0.05	0.271 ^{c} ± 0.01	91.201 ^{bc} ± 1.48	1.040 ^{b} ± 0.02
SH 7	5.576 ^{d} ± 0.16	0.733 ^{a} ± 0.02	413.242 ^{a} ± 8.54	0.272 ^{bc} ± 0	7.830 ^{a} ± 0.22	0.319 ^{c} ± 0.02	101.625 ^{ab} ± 2.21	0.865 ^{d} ± 0.03
SH 8	6.254 ^{bcd} ± 0.04	0.762 ^{a} ± 0.02	460.527 ^{a} ± 12.59	0.292 ^{b} ± 0	6.187 ^{bc} ± 0.33	0.348 ^{c} ± 0.01	88.458 ^{c} ± 0.39	0.946 ^{c} ± 0.01
F	4.77	52.76	74.22	21.33	21.33	21.33	12.45	20.50
p value	***	***	***	***	***	***	***	***

Notes: The columns with the same letter are not significantly different at P<0.05. The *** symbol next to each p-value in the table indicates that the differences between the sites are highly significant at p < 0.001.

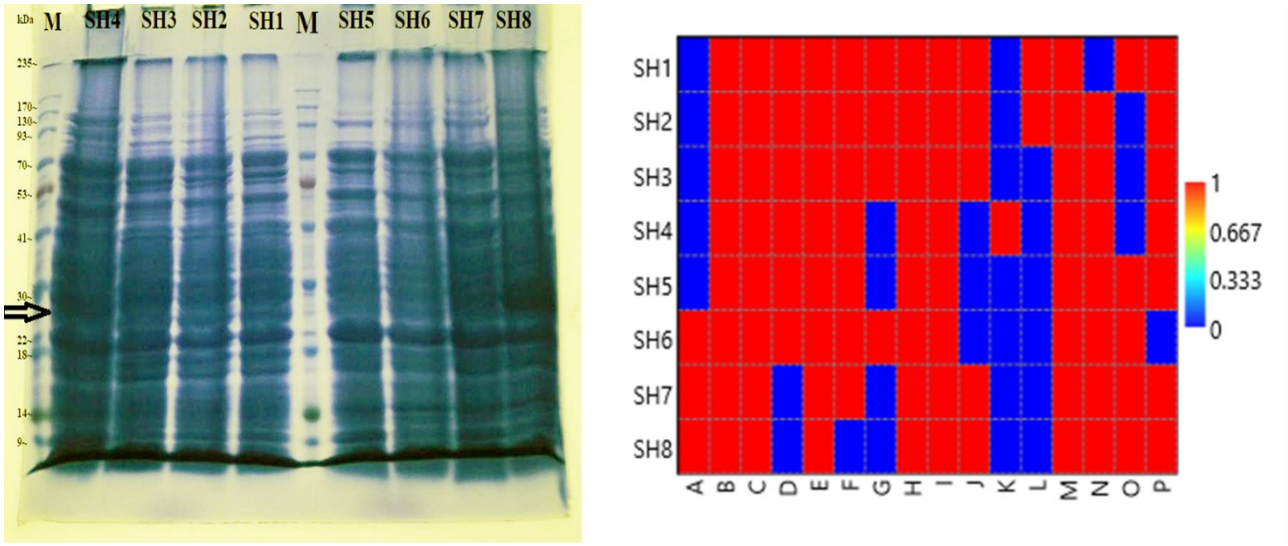
sites and the second group included SH4, SH5 and SH6 sites. The 2nd subcluster contained only one group included accessions of SH7 and SH8 sites Figure (4-a).

PCA scatter plot illustrate the relationships between the studied accessions and divided them into three subgroups, which were the same as those in the clustering analysis Figure (4-b). Similarity coefficient between the studied amaranth accessions measured at 96% as high similarity index between accessions SH2 and SH3 and measured at 72% as low similarity index between accessions SH4 and SH8; this result is illustrated in Table (5). The number of polymorphic bands calculated as 16 bands, number of monomorphic 6 bands, number of unique bands was 1 band of molecular weight 28 kDa for SH4 (Fig 3). Matrix plot constructed using the Past-pc based on Scoring and coding of SDS-PAGE of protein banding pattern to show variation among studied amaranth accessions (table 6 and fig 3-b), number of non-unique bands were 9 bands and degree of polymorphism calculated as 62.5% as shown in Table (6).

Discussion

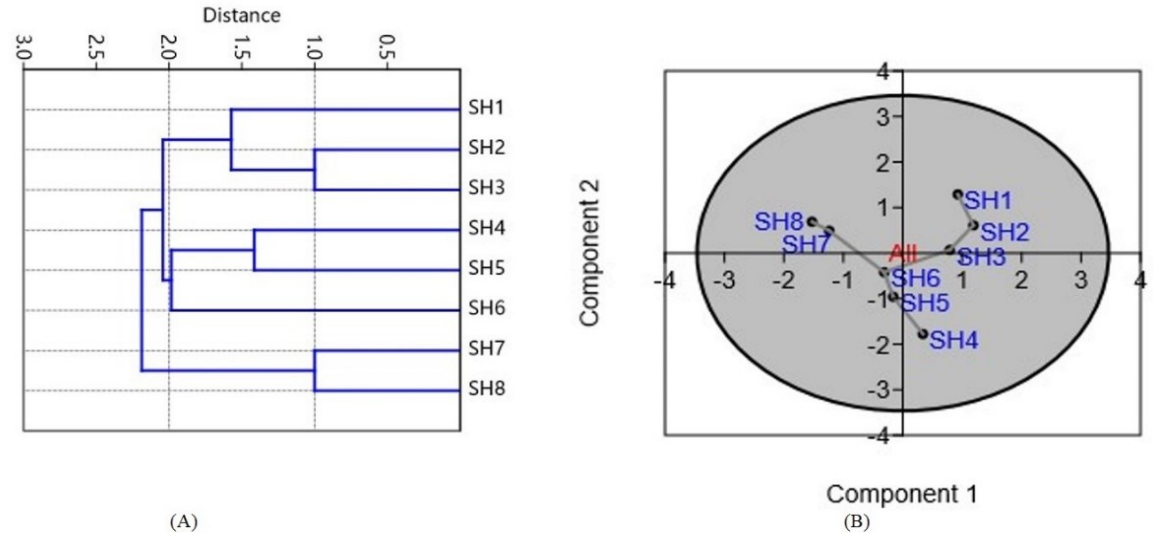
There are many factories of different specializations along the Ismailia canal in Egypt, and these represent multiple sources of pollution. These industries pose a great danger to the environment because they produce effluents, fumes and solid wastes contain heavy metals. A critical evaluation methods for selecting plants that are effective in soil remediation and tolerant to high levels of heavy metals (23). *In-situ* studies are important to explore how native species respond to heavy metal stress. The current study investigated the ability of *A. viridis* to accumulate large amounts of many heavy metal. Some literatures recommended that wild amaranth can be used as a potential plant for phytoremediation in different contaminated regions of the world (7, 8, 9).

Certain plants exhibit resistance to high soil metal concentrations, even while their biomass is unaffected. The results of heavy metal accumulation and plant development without affecting the growth are consistent with results of sunflower, which is considered to be a phytoremediator of



(A) (B)

Figure 3. (A) SDS-PAGE profile of storage protein for the examined accessions (SH1-SH8) and (B) matrix plot constructed using the Past-pc based on Scoring and coding of SDS-PAGE of protein banding pattern where code 1 indicate presence of band and code 0 indicate absence of band.



(A) (B)

Fig. 4. (A) A dendrogram cluster and (B) PCA scatter plot constructed using the software PAST-pc 4.2 software on protein banding of the eight accessions by SDS-PAGE.

Table 5. Similarity coefficient among the studied amaranth accessions (SH1-SH8) based on protein banding using SDS-PAGE.

	SH1	SH2	SH3	SH4	SH5	SH6	SH7	SH8
SH1	1							
SH2	0.923	1						
SH3	0.881	0.961	1					
SH4	0.755	0.839	0.871	1				
SH5	0.839	0.839	0.871	0.909	1			
SH6	0.801	0.801	0.833	0.784	0.871	1		
SH7	0.801	0.801	0.833	0.784	0.871	0.833	1	
SH8	0.755	0.755	0.784	0.727	0.818	0.784	0.958	1

Table 6. Scoring and coding of SDS-PAGE of protein banding pattern, Polymorphic, unique, non-unique and monomorphic bands in the studied accessions of amaranth.

Accession Code	Band Number																Total number
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
SH1	0	1	1	1	1	1	1	1	1	1	0	1	1	0	1	1	13
SH2	0	1	1	1	1	1	1	1	1	1	0	1	1	1	0	1	13
SH3	0	1	1	1	1	1	1	1	1	1	0	0	1	1	0	1	12
SH4	0	1	1	1	1	1	0	1	1	0	1	0	1	1	0	1	11
SH5	0	1	1	1	1	1	0	1	1	0	0	0	1	1	1	1	11
SH6	1	1	1	1	1	1	1	1	1	0	0	0	1	1	1	0	12
SH7	1	1	1	0	1	1	0	1	1	1	0	0	1	1	1	1	12
SH8	1	1	1	0	1	0	0	1	1	1	0	0	1	1	1	1	11
Parameter	Monomorphic bands				Polymorphic bands				Unique bands			Non-unique bands					Polymorphism %
Result	6				10				1			9					62.5 %

heavy metals such as Cd, Cu, Ni, Pb and Zn and has high biomass production (24). It was found that *Ocimum basilicum* L. can accumulate high amounts of Cd in different plant parts under the effect of EDTA without affecting the plant biomass (25). Moreover, the tolerance of phenological parameters in *Arundo donax* individuals to heavy metals was demonstrated (26).

The presence of different heavy metals (Cd, Cu, Ni, Pb and Zn) in *A. viridis* parts, such as shoots and roots defines successful phytostabilization and phytoextraction of these elements. This trend which helped *A. viridis* thrives in contaminated soils. Amaranth can accumulate and tolerate heavy metals due to its high antioxidant enzymatic and non-enzymatic defense system that protected amaranth under stress (27).Therefore, plants have the ability of adaptation to heavy metal toxicity, get rid of reactive oxygen molecules, and increase plant metabolism (11). One of the various methods for the phytoremediation of metal-contaminated soils is phytoextraction, which involves the absorption and storage of heavy metals in plant shoots and their removal from the treated area through plant organs (28). This requires the contaminants must be absorbed by plant roots and transferred to plant stems (29). Metals absorbed by amaranth would either be transferred by xylem vessels to the aerial parts of the plant or directed to the root tissues through phytostabilization processes. Adsorption, precipitation and complexation were some of the mechanisms used by the roots of phytostabilizers (30). In this investigation, amaranth showed higher BCF values of the estimated heavy metals at all sites. The larger surface area of the shoots could be the cause of excessive metal concentrations in the plants. The results of BCF values in amaranth are consistent with study of Qureshi et al (31). Additionally, the amaranth shoots showed greater TF values for Zn which made them phytoextractors from the soil, whereas their lower TF values for Cd and Fe made them phytostabilizers (32). The BCF and TF values suggested that the elm was a good candidate for the phytostabilization of Ni (33).

The relationship between protein content and heavy metal phytoremediation in plants is complex and multifaceted (34). Proteins play a crucial role in various physiological and biochemical processes within plants, including metal uptake, transport, detoxification and tolerance mechanisms. The presence of metals can induce the synthesis of specific proteins, such as metallothioneins and phytochelatins, which help in metal sequestration and detoxification. These metal-binding proteins assist in reducing the toxicity of heavy metals by forming stable complexes, preventing their translocation to sensitive plant tissues (35). Various Studies have shown that sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) can be used to assess the difference and similarity between plants (Schwartz et al., 1995). The variation in number of bands of protein may be caused by the harmful effects of reactive oxygen compounds, which cause lipid peroxidation and protein fragmentation or by heavy metal stress (36). Plants contain compounds inducing the antioxidant activity that can prevent the formation of reactive oxygen molecules and possibly lower oxidative cell damage (37). The current study clarified that the protein expression in various sites was significantly different from

each other depending on the number of protein bands. The result of the examined amaranth accessions revealed a total number of 16 bands with a large difference between some of the specific/unique bands used to differentiate accession from another, where the most prominent unique band was present in the SH5 accession, which had the highest TF value of Zn (1.94) and also both a dendrogram and a PCA blot showing the relationship between both the SH4 and SH5 accessions, where they both locate in the same subgroup and also have the same band number of 11 bands which have the least number of bands in compare to other accession and the reduction of protein content may be due to induction of lipid peroxidation and proteins fragmentation as a result of toxic effects of reactive oxygen species (ROS) (18), where the levels of heavy metal in both accessions have the highest concentration of Ni compared to other sites, which clarifies that under heavy metal stress, proteins were biosynthesized by amaranth.

Conclusion

Eight sites were selected for the study, representing multiple sources of pollution and producing different types of heavy metals. *A. viridis* was strongly present in these sites and showed significant variations in the morphological parameters. This confirms its ability to clean the soil it grows in as well as reduce the risk of heavy elements in order to preserve the environment. Values of BCF and TF recorded in *A. viridis* confirmed the plant's potential for phytostabilization and phytoextraction. Amaranth is an effective phytostabilizer for Cd, Fe and Ni and a phytoextracting agent for Zn and this has been confirmed also due to the variation in number of bands in the protein profile at different sites. Consequently, *A. viridis* is a very promising accumulator of heavy metals and can be used safely in phytoremediation techniques.

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Authors' contributions

Authors will declare the contribution of each author like SAI and MA conceived and designed the study. SAI and MA executed the experiment and analyzed the sera and tissue samples. All authors interpreted the data, critically revised the manuscript for important intellectual contents and approved the final version.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

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