Performance of purslane (*Portulaca oleracea*) in nickel and cadmium contaminated soil as a heavy metals-removing crop

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Abstract

Specific plants can remove heavy metals from the soil and contribute to pollution remediation in cropping systems. Determining the level of highest heavy metals that a super-accumulator crop can withstand without reducing its yield is important for management. The objective of this study was to investigate the heavy metal-removing capacity of purslane by studying different stress criteria and by tracking its nickel and cadmium removal from germination to harvest. Therefore, pot experiments in outdoor condition were performed by heavy metal levels including nickel (0, 30, 60, 120 mg/kg) and cadmium (0, 10, 20, 40 mg/kg) in two seasons. The results of this research showed that different levels of nickel and cadmium had significant effects on the morphological and physiological characteristics of purslane and increased heavy metals concentration decreased significantly these characteristics. Comparisons of mean shoot and root dry weight and extraction percentage showed that the highest level belonged to the control plants while the lowest level was observed in the plants under combined treatment of nickel (120 mg/kg) and cadmium (40 mg/kg) and the single treatment of cadmium (40 mg/kg). Perhaps heavy metals by their effect on dry and fresh matter made negative influence on extraction. Furthermore, the toxic properties of cadmium were more than nickel and decreased most of the measured characteristics.

Keywords: extraction; morphology; phytoremediation; soil pollution

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Introduction

Heavy metal pollution of agricultural soils is a major environmental problem that can affect plant productivity, food quality, and human health (Peralta et al., 2009). In line with the present day ecological and toxicological data obtained by Dutch ecologists, heavy metals/metalloids form the following succession according to their hazard degree in soils: Se > Tl > Sb > Cd > V > Hg > Ni > Cu > Cr > As > Ba (Vodyanitskii, 2016). Cadmium (Cd) is a heavy metal that is very toxic for plants, animals, and humans and can cause kidney damage, impair skeletal and reproductive systems, and other health problems (Singh et al., 2010; Taghipour et al., 2013; Yadegari and Karimpoor, 2010). It was reported to make disorder in uptake and transition, nutrient deficiency, and prevention of chloroplast composition (Ghani and Vahid, 2007), decreasing

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weight, root length, and leaf area (Murifah, 2008), and hydraulic conduction (Sahmurova et al., 2010). High levels, cadmium (up to 500 µM), has negative effects on plants growth and sprouting depending to variety, plant organ, essential elements required by the plant, time of planting, and poisonous emergence time of cadmium (Goncalves et al., 2009).

Nickel (Ni) is another heavy metal that has no toxic effect at low concentrations but in higher dosage it causes many destructive symptoms (Baycu et al*.,* 2006).

The use of traditional remediation technologies such as excavation and chemical leaching of metals is expensive and in most cases unfeasible (Baycu et al*.,* 2006; Karami and Zulkifili, 2010). Phytoremediation is an environmentally friendly technique to cope with the pollution of heavy metals of agricultural soils. Crop species differ greatly in their growth response when removing heavy metals from the soil. Some glycophytes can be intercropped with heavy metal-sensitive fruit trees to improve the tree growth; such sustainable utilization is actively promoted. Purslane (*Portulaca oleracea*) belonging to Portulacaceae family is a promising crop for regions polluted with heavy metals (Petropoulos et al., 2016). Researchers and nutritionists have suggested this plant as a potential vegetable crop for human consumption due to its nutritional and pharmaceutical importance. It is one of the richest vegetable sources of omega-3 fatty acids, a-linolenic acid, and glutathione (Alam et al., 2014; Uddin et al*.,* 2012; Osma et al., 2014). Purslane is highly adaptable to various stress environments and this characteristic gives purslane competitive advantages over many other cultivated crops (Yazici et al*.,* 2007; Salehi et al*.,* 2008; Dkhil et al., 2011; Cai et al., 2015). Therefore, purslane could be grown in regions moderately polluted by heavy metals to potentially augment its production of bioactive components which in turn probably can be harnessed for human health benefits (Thanh et al., 2015). Tiwari et al. (2008) reported purslane as hyper accumulator of Cd, Cr, and As. Therefore, plant tissues that are intended for human consumption should be thoroughly examined for their heavy metal content. To evaluate the heavy metal (Ni and Cd) removing capacity of purslane, this research was performed in two seasons measuring morphological and physiological characteristics such as shoot and root dry and fresh matter, extraction percentage, and nickel and cadmium accumulation of plant and soil.

Materials and Methods

Pot experiments in outdoor condition were established in 2015 and 2016 at Shahrekord (50°56/ E 32°18/ N) South Western Iran. The elevation of the region is 2060 m above sea level, annual precipitation is 650 mm, average minimum, maximum, and annual temperatures are -31 C°, 27 C°, and 12 C°, respectively. The soil physical and chemical properties are shown in Table 1. Heavy metal concentrations were below the United States Environmental Protection Agency's (UAEPA) maximum permissible limits. The required nutrients were added to the soil after the soil test. Seeds were sown in pots in the outdoor condition on May 22, 2015 and May 20, 2016. Treatments were cadmium (0, 5, 20, 40 mg/kg dry soil) from $K_2Cr_2O_7$ reference and Nickel (0, 20, 60, 120 mg/kg dry soil) from Ni $(NO_3)_2$ reference. For soil preparation, a sample was prepared from the soil of the field desiccated in air with a hammered plastic sheath and passed through a 2 mm sieve. The soil collected under the sieve was used for soil analysis. After analysis, soils were contaminated with heavy metals in the form of potassium dichromate and nickel nitrate,

Table 1
Some physical and chemical properties of soil in the experiment

Year	Texture	E.C ds.m^{-1}	N_{total} %	O.C.	pH	K	P	Zn	Mn	Fe	Cu	Cd	Ar	Pb	Ni
						mg.kg^{-1}									
1	Loam	8.19	0.12	0.3	7.16	795	50.1	1.02	12.48	8.09	1.38	0	0	0	0
2	Loam	7.9	0.11	0.2	7.1	781	49.8	1.1	11.1	8.1	1.2	0	0	0	0

water-soluble and spray-applied to soil and mixed with soil. To balance, the treated soil was left to rest for at least two weeks, exposed to more aging and drying periods where possible, and allowed reactions between heavy metals and soil to occur and to achieve contamination closer to the natural conditions (Davari et al., 2010).

The experiments were arranged in a randomized complete design with three replications. Dry and fresh weight of shoot and root, extraction of shoot, nickel and cadmium accumulation in shoot and soil after harvesting were measured. The extracts were prepared following methods described by Crozier et al. (1997) with slight modifications. Two grams of dry powdered purslane sample was weighed out in a 100 mL conical flask and 20 mL of methanol was added and left for 2 h in a water bath shaker with 100 rpm at 40 ± 1 $^{\circ}$C. The filtrate was separated from the residue by filtering through a filter paper (Whatman No. 1). The residue was re-extracted again with fresh solvent according to the procedure mentioned above. The filtrates were pooled and surplus methanol was then evaporated off under reduced pressure using a rotatory evaporator (Buchi Rotavapor R-210, Switzerland). The concentrated extract was then stored at -20 ± 1°C for analysis.

For measurement of absorbable cadmium and nickel in soil, the decoction with de ethylene three amine pentad acetic acid method was used and after shaker similar previous method the data by ppm unity was registered (Diaconu et al., 2012). Heavy metals in shoots were obtained by wet digestion method through atomic adsorption equipment and a graphic furnace (Diaconu et al., 2012; Davari et al., 2010). By using a sieve No 230, particles smaller than 63 micron and which were tiniest grains in the study were separated from soil samples. All data were subjected to ANOVA using SAS, and the means were separated using L.S.D. test at $P<0.05$.

Results

The root and shoot dry weights of purslane mainly decreased as the pollution of heavy metals increased; these decreases were more than 400% for Cd40 × Ni120 in the dried matter at harvest (Tables 3 and 4). Dry mass responses were statistically significant and the expected damage of heavy metal was evident mostly at the harvest in the developed plants. At harvest, tissue Cd and Nickel were significantly higher ($p<0.01$) with higher heavy metals levels, up to 400% compared to controls (Tables 3 and 4).

Effects of heavy metal pollution on plant growth and extract production characteristics are given in Tables 2-4. Results showed significantly different effects induced by cadmium and nickel on growth parameters of purslane. Plants also showed different responses due to heavy metals with different concentrations. Pollution with cadmium significantly decreased dry and fresh weights of shoot and root ($p<0.01$). Pollution effects with cadmium increased more than nickel, where the least values for dry and fresh weight of shoot and root were observed by high concentration of cadmium. Low concentration of nickel was in similar group with control but high concentrations of the two heavy metals had the maximum effect on decreasing shoot and root dry

Table 2
Combined analysis of mean square of shoot dry weight, shoot fresh weight, root dry weight, root fresh weight, extraction percentage, Nickel of plant and soil, Cadmium of plant and on soil, in purslane plants affected by several heavy metals

Source of variation	Degree of freedom	Shoot dry weight	Shoot fresh weight	Root dry weight	Root fresh weight	Extraction percentage	Nickel of plant	Nickel of soil	Cadmium of plant	Cadmium of soil
Year(Y)	1	0.4^{*}	41.1^{ns}	0.31^{*}	0.23^{ns}	0.33^{*}	3.2^{*}	2.6^{*}	0.5^{*}	0.65^{*}
R/Y	4	0.4^{*}	14.2^{ns}	0.35^{*}	0.18^{ns}	0.34^{*}	2.1^{*}	1.5^{*}	0.28^{*}	0.4^{*}
Nickel (Ni)	3	2056.3^{**}	963962.4^{**}	20.5^{**}	187.5^{**}	7.5^{**}	615.1^{**}	2715.1^{**}	13.8^{**}	62.4^{**}
Cadmium (Cd)	3	9954.8^{**}	3328468.2^{**}	145.5^{**}	1412^{**}	86.7^{**}	30.8^{**}	15.1^{**}	1338.1^{**}	40.7^{**}
Ni × Cd	9	2972.2^{**}	1337000.1^{**}	17.4^{**}	177.7^{**}	14.1^{**}	20.7^{**}	10.9^{**}	6.2^{**}	26.3^{**}
T (Ni, Cd) × Y	15	57.1^{**}	40.1^{ns}	0.14^{*}	0.2^{*}	0.14^{*}	0.16^{*}	1.2^{*}	0.23^{*}	0.32^{*}
Error	60	0.08	34.6	0.06	0.09	0.065	0.8	0.56	0.11	0.15
Coefficient of Variation		0.74	1	2.4	1	2.9	5.9	5.3	3.5	6.6

weight. The highest shoot and root dry weights were obtained from control plants. Plants polluted with heavy metals alone specially nickel, had greater yield and extraction than combination of two heavy metals. Combined pollution with cadmium and nickel resulted in a significant decrease ($p<0.01$) over nickel or cadmium alone for most of the measured characters (Table 2). Plants contaminated with two heavy metals showed less dry and fresh matter compared to plants polluted with cadmium or nickel alone but the amount of nickel and cadmium in shoot and soil of plants treated with combination of two heavy metals was the highest (Tables 3 and 4). The highest dry and fresh shoot and root weights were achieved by control plants; however, these were similar with the plants polluted with the lowest concentration of heavy metals specially Ni. Cadmium and nickel contents of the shoot and soil after harvesting were affected by the amount of

Table 3
L.S.D comparisons of measured characters of Purslane that affected by several heavy metals in the first year.

Treats	Shoot dry weight (g/plant)	Shoot fresh weight (g/plant)	Root dry weight (g/plant)	Root fresh weight (g/plant)	Extraction (%)	Nickel of plant (mg/kg)	Nickel of soil (mg/kg)	Cadmium of plant (mg/kg)	Cadmium of soil (mg/kg)
Cd0×Ni0	502±10.2	1572±22.4	66.5±5.7	177.5±8.2	17.1±1.1	0±0	0±0	0±0	0±0
Cd0×Ni20	382±8.8	986.5±20.1	54.4±4.7	149.3±6.9	15.5±0.9	11.7±1.1	5.4±1.1	0±0	0±0
Cd0×Ni60	320±7.5	945.3±18.2	49.4±3.5	147.9±5.9	14.3±0.8	43.1±2.4	12.4±2.2	0±0	0±0
Cd0×Ni120	295±6.9	887.4±15.5	33.8±2.5	90.2±3.2	13.9±0.7	85.4±2.7	18.7±2.2	0±0	0±0
Cd5×Ni0	312±10.3	949.4±12.7	59.8±3.2	172.3±2.5	13.8±0.6	0±0	0±0	8.2±0.8	6.6±0.5
Cd5×Ni20	281±9.6	852.2±10.4	50.2±2.2	142.1±7.5	7.6±0.4	10.1±1.3	6.9±1.1	7.95±0.8	6.5±0.3
Cd5×Ni60	195±8.8	498.9±11.1	39.7±3.1	117.8±6.9	5.5±0.3	33.1±2.2	11.4±3.3	7.8±0.7	6.3±0.2
Cd5×Ni120	124±9.7	375.9±10.1	27.7±2.1	80.2±4.2	5.1±0.2	68.6±3.1	25.9±2.7	7.82±0.8	6.3±0.1
Cd20×Ni0	241±9.5	733.4±11.1	39.5±2.8	119.4±5.5	9.2±0.6	0±0	0±0	15.9±1.1	12.2±1.1
Cd20×Ni20	185±8.6	555.9±9.9	41.2±1.5	111.4±3.2	5.9±0.4	9.9±1.3	11.4±1.3	14.4±0.9	11.9±0.9
Cd20×Ni60	121±7.7	382.6±8.8	31.2±1.6	80.4±2.9	5.3±0.2	29.7±1.8	15.8±1.5	14.2±0.8	11.8±0.8
Cd20×Ni120	99.6±9.2	299.6±8.9	22.1±0.9	51.3±1.6	5.1±0.1	59.4±2.7	25.4±4.1	14.1±0.8	11.9±0.7
Cd40×Ni0	58±2.2	178.9±5.5	18.8±1.1	32.2±2.1	4.73±0.1	0±0	0±0	29.6±2.2	21.9±1.8
Cd40×Ni20	112±7.8	336.7±8.9	30.1±1.6	76.7±3.3	6.6±0.2	5.7±0.9	14.4±2.4	28.2±1.1	21.5±1.5
Cd40×Ni60	88.5±8.3	278.5±9.2	24.3±1.9	69.3±2.9	5.6±0.3	18.9±0.9	29.5±1.6	27.6±1.5	20.5±1.1
Cd40×Ni120	62.2±5.8	199.3±6.9	18.1±1.6	33.5±3.2	4.9±0.1	45.1±2.1	33.9±2.4	27.1±1.1	20.3±0.9

Table 4
L.S.D comparisons of measured characters of Purslane that affected by several heavy metals in the second year.

Treats	Shoot dry weight (g/plant)	Shoot fresh weight (g/plant)	Root dry weight (g/plant)	Root fresh weight (g/plant)	Extraction (%)	Nickel of plant (mg/kg)	Nickel of soil (mg/kg)	Cadmium of plant (mg/kg)	Cadmium of soil (mg/kg)
Cd0×Ni0	521±9.9	1613±20.1	68.8±4.4	183.8±6.5	18.2±0.9	0±0	0±0	0±0	0±0
Cd0×Ni20	395±8.5	999.5±18.1	56.6±3.3	155.5±6.5	15.7±0.8	12.2±1.1	6.4±1.1	0±0	0±0
Cd0×Ni60	331±8.2	975.7±15.2	52.2±3.3	161.1±5.1	14.8±0.7	45.2±2.4	14.4±2.2	0±0	0±0
Cd0×Ni120	301±7.2	899.4±14.5	35.6±2.3	94.6±3.6	14.3±0.6	86.1±2.4	18.5±1.1	0±0	0±0
Cd5×Ni0	320±9.4	966.4±11.1	62.1±2.8	179.2±2.1	13.9±0.6	0±0	0±0	8.5±0.5	7.1±0.4
Cd5×Ni20	288±8.5	866.6±9.9	52.5±2.7	149.1±4.4	7.8±0.2	12.2±1.2	7.5±1.9	8.3±0.4	6.9±0.2
Cd5×Ni60	202±8.2	514.2±10.6	42.2±2.9	122.2±6.2	5.9±0.2	35.5±2.6	12.7±1.4	8.1±0.5	6.8±0.3
Cd5×Ni120	126±8.7	402.4±9.5	29.1±2.8	82.2±2.2	5.5±0.1	69.3±3.9	27.7±1.1	7.95±0.6	6.7±0.4
Cd20×Ni0	255±6.6	755.5±10.2	41.9±3.3	123.3±3.5	9.5±0.3	0±0	0±0	16.2±0.9	13.4±0.9
Cd20×Ni20	191±9.2	566.6±8.5	44.5±3.6	114.5±5.2	6.2±0.2	10.1±1.5	11.1±1.4	15.8±0.8	13.2±0.8
Cd20×Ni60	125±6.5	399.5±8.9	33.5±3.1	85.5±2.5	5.5±0.2	31.2±1.9	15.5±1.3	15.7±0.7	12.9±0.7
Cd20×Ni120	101±5.5	314.8±11.4	25.2±1.1	55.2±1.5	5.3±0.1	60.1±2.2	25.9±2.2	15.3±0.5	12.8±0.8
Cd40×Ni0	61±3.5	185.2±4.3	19.7±1.3	35.9±2.9	4.9±0.1	0±0	0±0	30.2±2.4	22.1±1.1
Cd40×Ni20	114±4.5	347.5±7.5	32.2±2.2	79.9±3.4	6.4±0.2	6.5±1.1	14.1±1.1	29.5±0.9	21.9±1.15
Cd40×Ni60	89.2±7.9	284.5±6.9	26.5±2.4	71.4±2.4	5.7±0.1	19.5±1.8	30.2±1.5	28.8±0.8	21.7±0.9
Cd40×Ni120	66.5±6.5	204.6±6.6	19.2±1.5	36.7±3.7	5.1±0.1	49.1±5.5	33.5±1.6	28.1±0.9	21.6±0.8

heavy metals that the plants were treated with. Toxic effects of cadmium on plants were more than nickel (Hushmandfar and Moraghebi, 2011). Comparison of means showed that control plants and the plants treated with lower concentrations of Cd were similar in most characteristics (Tables 3-4). The correlation between tissue cadmium and nickel concentration was significant ($p<0.01$). Shoot Ni and Cd concentrations were significantly ($p<0.01$) higher at elevated heavy metals levels, 85.4- 86.1 mg/kg and 29.6-30.2 mg/kg compared with 0 mg/kg and 0 mg/kg in the controls in two seasons, respectively (Tables 3-4). Tissue Cd and Ni concentrations positively correlated ($p<0.01$). Interactions between heavy metal levels in soil and in plant tissue were significant ($p<0.01$). The dry weight of shoots and roots indicates the heavy metals tolerance of purslane. Accumulation of nickel and cadmium in root zone were significantly ($p<0.01$) higher in the heavy metal-treated plants compared to controls. By increasing Cd or Ni in plant materials, the extraction and weight of shoots and roots reduced (Ttables 5-6).

Discussion

There were significant differences between the extraction and measured characteristics of the various samples. Also, a positive correlation was observed between the nickel contents of the soil and dry shoot weight of the plants. Contents of the cadmium and nickel in soil significantly correlated with the cadmium and nickel in plant tissue, and correlation coefficients between the extraction content and root or shoot fresh or dry matter suggested that the extraction content of plants usually had a positive effect on the extraction content of the other tissues, and cadmium and specially nickel had negative effects on extraction content (Tables 5-6). Mobility of nickel resulted in higher accumulate of this metal in shoot tissue and at higher concentrations of

Table 5
Results of Pearson correlation coefficient between characteristics in purslane plants affected by several heavy metals in the first year

Characters	Root fresh weight	Root dry weight	Nickel of plant	Nickel of soil	Cadmium of plant	Cadmium of soil	Extraction percentage	Shoot dry weight	Shoot fresh weight
Root fresh weight	1								
Root dry weight	0.99**	1							
Nickel of plant	-0.81 **	-0.91**	1						
Nickel of soil	-0.77 **	-0.66 **	0.86**	1					
Cadmium of plant	-0.78**	-0.78**	0.13 ns	0.04 ns	1				
Cadmium of soil	-0.7**	-0.71**	0.25 ns	0.25 ns	0.9**	1			
Extraction (%)	0.94**	0.92**	-0.88**	-0.77**	-0.75**	-0.68**	1		
Shoot dry weight	0.91**	0.9**	-0.65**	-0.88**	-0.58**	-0.51**	0.93**	1	
Shoot fresh weight	0.84**	0.82**	-0.59**	-0.77**	-0.49**	-0.43**	0.89**	0.98**	1

Table 6
Results of Pearson correlation coefficient between characteristics in purslane plants affected by several heavy metals in the second year

Characters	Root fresh weight	Root dry weight	Nickel of plant	Nickel of soil	Cadmium of plant	Cadmium of soil	Extraction percentage	Shoot dry weight	Shoot fresh weight
Root fresh weight	1								
Root dry weight	0.88**	1							
Nickel of plant	-0.8 **	-0.87**	1						
Nickel of soil	-0.65 **	-0.75 **	0.69**	1					
Cadmium of plant	-0.85**	-0.84**	0.13 ns	0.04 ns	1				
Cadmium of soil	-0.9**	-0.81**	0.25 ns	0.25 ns	0.77**	1			
Extraction percentage	0.9**	0.8**	-0.9**	-0.8**	-0.8**	-0.77**	1		
Shoot dry weight	0.95**	0.94**	-0.66**	-0.87**	-0.64**	-0.5**	0.71**	1	
Shoot fresh weight	0.89**	0.85**	-0.65**	-0.85**	-0.66**	-0.55**	0.85**	0.99**	1

ns,* and **: Non significant, significant at the 5% and 1% levels of probability, respectively cadmium, nickel inhibited to transition to aerial parts of plants (Marschner, 1995; Al-Rashdi and Sulaiman, 2013). In the present study, Ni and Cd concentrations in plant tissues were high, probably because of continuous contact with heavy metals in the root zone. Findings showed that roots of purslane were more tolerant than shoots which is consistent with the findings of Baycu et al. (2006) and Shi and Cai (2009) who studied plants exposed to heavy metal pollution and showed that in different plants shoots or roots accumulated a higher concentration of cadmium. They also showed that at low concentrations of nickel, purslane plants did not have any response to heavy metals but at higher concentrations decrease in yield parameters and extraction was significant (Tables 3-4). Nickel and cadmium contents of plant and soil negatively affected shoot and root dry and fresh matter. Combination of heavy metals had more effects, decreasing dry and fresh matter in shoot and root and extraction percentage compared to single treatment of each heavy metal (Tables 3-4). The amounts of cadmium and nickel in soil at harvest were variable but at higher concentrations of these heavy metals, more cadmium and nickel were observed (Tables 3-4). However, Cd40×Ni0 resulted in greater pollution than other treatments. These results showed a more toxicity of cadmium than nickel. The synergetic effect of two heavy metals were previously observed by other researchers (Sahmurova et al., 2010; Rahman Khan and Mahmud Khan, 2010; Shi and Cai, 2009). It seems that the subterranean structures result in the highest aggregation of heavy metals in plant shoots and roots. This is in agreement with previous reports demonstrating the effects of cadmium as more destructive than nickel on growth and development of plants (Pandey and Pandey, 2009; Hushmandfar and Moraghebi, 2011; Sarma, 2011; Abou Auda et al*.,* 2011).

Results revealed that combined application of cadmium and nickel decreased the amount of dry matter and therefore extraction of plant was diminished (Tables 3, 4). As a result, gross average of extraction percentage decreased from 17.1-18.2% for control plants to 4.73-4.9% for those polluted with Cd40×Ni0. Results showed that cadmium had much destructive effects on the measured characteristics than nickel. This difference is probably attributable to metabolic production as well as higher ability for enzyme production at low concentration of Ni (Houston, 2007).

Results of this study suggest that nickel with low concentration of cadmium more percolate to plant tissue but more than 20 mg/kg cadmium in soil, transporting of nickel to plant tissue was decreased. Results also indicate that the extraction content of *Portulaca oleracea* L. was significantly affected by differences in heavy metals properties of the soil. In growing seasons of purslane 29.6-30.2 mg/kg cadmium and 85.4-86.1 mg/kg mg/kg nickel were removed. The most destructive treatments were Cd40×Ni0 and Cd40×Ni120. In addition, the present study also demonstrates that the extraction content of *P. oleracea* was affected by the mobility of nickel and toxicity of cadmium transmitted with nickel to plant tissues. This study provides useful information regarding the effect of heavy metals on the content of extraction in *P. olerace* and the phytoremediation potential of purslane.

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