The effect of heavy metals (Cd and Ni) on growth, yield and other characters of *Portulaca oleracea* L.

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**ABSTRACT**

Purslane is a local plant in Iran that this plant can easily breed and is also highly tolerant against lack of water; it can be regarded as a hyper accumulator of heavy metals. To study the effect of the nickel and cadmium on growth, yield and other characters of *Portulaca oleracea* L. a pot experiment was carried out in a completely randomized factorial design with three replications in the field of Azad University of Shahrekord, Iran, in the spring and summer of 2011 and 2012. Treatments of this experiment included different levels of nickel (0, 20, 60, 120 mg/kg) and different levels of cadmium (0, 5, 20, 40 mg/kg). The results of this research showed that different levels of nickel and cadmium have a significant effect on the morphological and physiological characters of *Portulaca oleracea* L. and along with increasing the concentration of the heavy metals, these characters significantly decreased in comparison with the control plants. In classifying the averages of shoot and root dry matter and percentage of the extract, and Cd and Ni of soil and shoot, the highest amount belonged to the control plants and the lowest amount to the plants under combined treatment of nickel (120 mg/kg) and cadmium (40 mg/kg) and the single treatment of cadmium (40 mg/kg). In study of phonological trend and Duncan's multiple range test, we saw the different groups. Perhaps heavy metals by effect on dry and fresh matter made negative effect of essence. Furthermore the toxic properties of cadmium are more than nickel and decreased most of measured characters.

**INTRODUCTION**

Heavy metals pollution of agricultural soils is a major environmental problem that can affect plant productivity, food quality and human health (Peralta et al., 2009; Alloway, 1990b; Jorgensen, 1991; Kabata-Pendias and Pendias, 2001; McGrath et al., 2002). One of them is Cadmium that is very toxic for plants, animals and humans and can cause kidney damage, impair skeletal and reproductive systems and other health problems (Shanker et al., 2005; Houston, 2007; Singh et al., 2010 + Taghipour et al., 2013; Yadegari and Karimpoor, 2010). It made disorder in uptake and transition (Robinson et al., 2000 + Wu and Zhang, 2002 + Zhang et al., 2002), nutrient deficiency (Epstein and Bloom, 2005), prevention of chloroplast composition (Ghani and Vahid, 2007), decreasing of weight, root length and leaf area (Muirfah, 2008), hydraulic conduction (Sahmurova et al., 2010), water uptake, plant growth (Schutzendubel et al., 2001), leaf chlorosis and root necrosis (Vassilev et al., 2005). A few amount of cadmium depending upon kind of plant don’t disturb in growth. Conversely, at high amount (up to 500 μ mol), has negative effect on plants growth and sprouting depending to variety, plant organ, essential elements required plant, time planting and poisonous emergence time of cadmium (Goncalves et al., 2009). Nickel is another heavy metals that have no toxic effect in low concentration but in higher dosage have many destructive symptoms (Baycu et al., 2006; Smith, 1996). Nickel holds a special place among the heavy metals. Unlike Cd, Pb, Hg, Ag, and several other metals that are not the components of plant enzymes, Ni is a constituent of urease, and small quantities of Ni (0.01 to 5 g/g dry wt) are essential for some plant species. However, same as with other heavy metals, high Ni concentrations may turn toxic to plants (Seregin and Kozhevnikova, 2006). The use of traditional remediation technologies such excavation and chemical leaching of metals is expensive and in most cases unfeasible (Cunningham et al., 1995). A promising remediation technology for mildly heavy metal polluted soils is phytoextraction (Salt et al., 1995). However, the use of hyper accumulator plants for phytoextraction presents some challenges because these are mostly wild species with significant metal uptake but limited biomass production and provide no economic return (Baker et al.,
Purslane (Portulaca oleracea) is promising crop for pollution agriculture. In this regard, purslane is a reasonable selection due to its high nutritive and antioxidant properties as human food, animal feed and medical utilization. Purslane comprises more nutritive values than other vegetable due to its fatty acid, α-tocopherol, ascorbic acid, β-carotene and glutathione-rich shoots (Wenzel et al., 1990; Lasat, 2002; Cai et al., 2004; Salehi et al., 2008). With respect to purslane as a medical and valuable plant and because of low information regarding its physiological reactions under stress environment condition, doing this project is necessary. The current study aimed to evaluate the beneficial effect of purslane on phytoremediation in pollution areas that polluted by heavy metals.

Methodology:

Studies were conducted during the 2011 and 2012 growing seasons at the experiment station of College of Agriculture, Islamic Azad University Branch of Shahrekord (50°56' E 32°18' N) to investigate the effects of heavy metals (Cd and Ni) in different concentrations on growth, yield and other characters of Portulaca oleracea L. The soil was a silty clay loam (Table 1). The experimental design was a randomized complete block with three replications. Each pot was 10 kg capacity and under each pot, there was basin for inhibit of heavy metals output. Sixteen different heavy metals concentrations were employed at the beginning of sowing of plants:

Cd: 0, 5, 20 and 40 mg/ kg dry soil,
Ni: 0, 20, 60 and 120 mg/kg dry soil.

For pollution of heavy metals, measured of heavy metals fertilizer (cadmium nitrate and nickel nitrate) added to soil in solution format. Sowing of purslane was done manually, 0.5cm depth and densities of 10 plant m², on 22 May 2011 and 20 May 2012. Weeds were controlled by hand, when required. To record the developmental stages on a weekly basis, 3 plants of each treatment were monitored. The developmental stages were determined when one plant in each plot indicated that stage. The developmental stages were measured by GDD. GDD was calculated as:

\[
\sum \left( \frac{T_{\text{max}} + T_{\text{min}}}{2} - Tb \right)
\]

Where GDD is the growing degree day, Tb the threshold temperature for growth (°C), Tmin the minimum air temperature (°C), and Tmax is the maximum air temperature (°C). Tb of purslane was considered as 7°C (Kruk and Benech-Arnold, 1998). Due to the differences in maturity induced by the different heavy metals treatments, there were different harvest dates for plants in both years of the study. Harvesting was done manually by pulling the dry plant out of the soil and removing the roots. Important characters consisted of dry and fresh weight of shoot and root, essence of shoot, nickel and cadmium in shoot and soil after harvesting. Essence of harvested plants by Clevenger was measured. For measurement of absorbable cadmium and nickel in soil the decoction with “De ethylene three amine pentad acetic acid” method served and after shaker similar previous method the data by ppm unity was registered. The method of obtaining heavy metals in shoots is called wet digestion (W.D) in which vegetarian organs beside an acid mixture digested and the measurement of extract obtained by atomic absorption machine in such a way that called graphic furnace has been done. Most of methods done according to international standard book performed. The samples of soil and plant dried in an oven in 70°C. Then by using sieve number 230 particles which are smaller than 63 micron and are tiniest grains in this research separated from soil samples. By using agate mortar or silicate mortar, Soil and plant samples powered and homogenized. The reason for choosing for metals such as Cd and Ni in common investigations is considering presents of these elements in most output of the research region industry and the plenty of them incrust as well. The metal mobility coefficient is equal to heavy metal concentration on air organ divided by the same heavy metal concentration on root organ (Adamo et al., 2002; Videa et al., 2002 and APHA, 1995). The calculation of data obtained experiment has been done by statistic software such as SPSS, SAS, and Excel 2013. Means comparisons separated with Duncan’s multiple range test at P<0.05 level.

Results:

The effects of heavy metals pollution on plant growth and essence production characteristics are given in Tables 2-4 and Figures1-18. We found significant 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 (p < 0.01) decreased final LAI, dry and fresh weight of shoot and root. Pollution with cadmium decreased more than nickel, where the least values for dry and fresh weight of shoot and root were observed by high concentration of cadmium. Similar result was obtained for leaf area index from planting to harvesting (Fig 1-4). Low concentration of nickel was in similar group with control but high dosage of combination of two heavy metals had the most effectiveness on yield characters. The highest shoot and root dry matter were obtained from control plants that no have any heavy metals. Cd,Ni treatment
has the most destructive effect between treatments and therefore plants treated by this, in below GDD were
demised. Plants polluted with higher concentration of heavy metals which showed a 1300% decrease over
control (35 gr/plant shoot dry matter in Cd, Ni in contrast of 480gr/plant in control plants in second year). Plants
inoculated with heavy metals alone specially nickel, had greater yield and essence than combination of two
heavy metals. Probability, the maximum range for heavy metals tolerance was obtained in GDD=1000 (Fig3, 4).
Pollution of cadmium and nickel together resulted in a significant (p < 0.01) decrease over nickel or cadmium
alone for most of measured characters (Table 2). Similar pattern was also demonstrated for phonological
properties (Figures 3, 4). Plants contaminated by two heavy metals showed slower dry and fresh matter compared
to plants polluted by cadmium or nickel alone (Figures 7-10) but the amount of nickel and cadmium in shoot
and soil of plants treated by combination of two heavy metals was the most (Figures 11-18). Highest dry and
fresh shoot and root was achieved by control plants however these were similar with the plants polluted by
lower concentration of heavy metals specially Ni. The amount of cadmium and nickel in shoot and soil after
harvesting were affected by the amount of heavy metals that the plants polluted. Cadmium was more than nickel
had toxic effect in plants. Plants contaminated by upper concentration of heavy metals were falling and decrease
dry matter at an earlier time than other plants and therefore less essence percentage (Figures 3-6). The results of
correlation between characters were significant (p < 0.01). The amount of dry and fresh matter with essence
percentage had positive correlation and other hand cadmium and nickel per plant and soil after harvesting had
negative effectiveness (Tables 3, 4).

Discussion:
This work has shown the effects of heavy metals on the dry and fresh matter of shoot and root, essence
percentage, amount of accumulation of cadmium and nickel of shoot in purslane plants that polluted by nickel
and cadmium in two seasons. Purslane is believed to be a hyper accumulator due to the heavy metals. However,
in the present study, particularly in the final stage of growth, tissue Ni and Cd concentrations were always high,
probably because of continuous contact with heavy metals in the root zone. These findings showed that roots of
purslane were more tolerant than shoots and consistent with Baycu, et al (2006); Ardini et al., (1994); Peralta et
al., (2004); Shi and Cai (2009) and Fuentes et al.,(2006) who studied plants under polluted conditions and
showed that in different plants shoots or roots accumulated a higher concentration of Cd. They are showed that
in low concentration of nickel, purslane plants no have any responded to heavy metals but in higher density,
decreasing in yield parameters and essence was significant (Figures1-10 and Table 2). All plant factors except of
in Ni and Cd in plant and soil, we measured in this study were negatively affected by heavy metals. Combination
of heavy metals were able to decrease dry and fresh matter in shoot and root, essence percentage and final leaf
area index, more than single of each heavy metals (Table 2 and Figures 1-10). Amount of cadmium and nickel
accumulated by plants in various treatments was ranging from 0 to 30 mg/kg and 6.2 to 27 mg/kg respectively
(Figures13-14, 17-18) depending on the concentration of heavy metals used during two years of the study. The
amount of Cd and Ni in soil after harvesting was different too (Figures 11-12, 15-16). However, Cd,Ni showed
greater pollution efficiency than Cd or Ni. This results showed the more toxicity of cadmium than nickel and
also the synergist effect of two heavy metals were previously observed by other researchers (Prasad and Feritas,
2003; Ouzoundi et al., 1995; Manio et al., 2003; Prasad and Feritas, 2003; Sharma and Gaur, 1995; Baker et al.,
2000; Parida et al., 2003; Moya et al., 1993; Wojcik et al., 2005). Pollution of heavy metals resulted in worse
appearance of leaf area index which was translated into lesser shoot and root dry matter accumulation and
essence production (Figures 3, 4). It seems that the subterranean structures results the most aggregation of heavy
metals in plants, from roots to aerial parts in plants. Translocation of heavy metals make is less (Bose and
Bhattacharyya, 2008) and there are correlation between concentration of cadmium in leaves and rhizosphere
(Lorenz et al., 1997). This is in agreement with previous reports demonstrating the destructive effects of
cadmium is more than nickel on growth and development of plants (Pandey and Pandey (2009); Sanita di Toppi
and Gabbirelli, 1999; Lombi et al., 2000; Zhang et al., 2002; Houston, 2007; Hushmandfar and Moraghebi,
2011). The results revealed that application of cadmium and nickel together decrease the amount of dry matter
and therefore essence of plant was diminished (Tables 3, 4). As a result, gross average of essence percentage
decreased from 18% for control plants to 4.76% for those polluted with Cd,Ni. Our data showed that cadmium
had much worse effect on measured characteristics than nickel. This difference is probably attributable to
metabolic production as well as higher ability for enzymes production in low concentration of Ni (Seregin and
Kozhevnikova, 2006).

Conclusion:
The heavy metals-removing capacity of the glycophyte purslane and its high tolerance to Ni and Cd toxicity
makes it a promising crop in place of halophytes. It has a spreading habit that can be suited to intercropping with
fruit trees. Purslane had almost no yield reductions in low concentration polluted environments. Its short
vegetation period and all years round production in many arid and semiarid conditions can allow a continuous
Ni and Cd removal that may accumulate in the orchard soils during the irrigation seasons. Results of the present
study show that in one growing season of purslane about 30 mg/kg and 24 mg/kg for cadmium and nickel
respectively are removed. In this regard, if practical and manageable in agriculture, successive purslane
cultivations all year round will enable higher and continuous cumulative heavy metals removals. Moreover, the high concentration of nickel and cadmium after harvesting wholly can remove and therefore for after cropping, we have no problems. Our study showed that the high concentration that purslane plants can tolerant was 120 mg/kg for nickel and 40 mg/kg for cadmium and after these dosage, plants were demise.

Table 1: Some physical and chemical properties of soil for experiment (0 -30) cm.

<table>
<thead>
<tr>
<th>Year</th>
<th>Texture</th>
<th>E.C (ds.m⁻¹)</th>
<th>pH</th>
<th>K</th>
<th>P</th>
<th>Zn</th>
<th>Mn</th>
<th>Fe</th>
<th>Cu</th>
<th>Cd</th>
<th>Ar</th>
<th>Pb</th>
<th>Ni</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Loam</td>
<td>8.19</td>
<td>7.16</td>
<td>795</td>
<td>50.1</td>
<td>1.02</td>
<td>12.48</td>
<td>8.09</td>
<td>1.38</td>
<td>0.86</td>
<td>1.11</td>
<td>1.18</td>
<td>4.58</td>
</tr>
<tr>
<td>2</td>
<td>Loam</td>
<td>7.9</td>
<td>8.11</td>
<td>781</td>
<td>49.8</td>
<td>1.11</td>
<td>11.1</td>
<td>8.1</td>
<td>1.2</td>
<td>0.88</td>
<td>0.99</td>
<td>1.1</td>
<td>4.4</td>
</tr>
</tbody>
</table>

Table 2: Results of correlation between characters in purslane plants that are affected by several heavy metals in first year.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Degree of freedom</th>
<th>Root dry matter</th>
<th>Root fresh matter</th>
<th>Nickel of soil</th>
<th>Cadmium of plant</th>
<th>Cadmium of soil</th>
<th>Essence percentage</th>
<th>Nickel of soil</th>
<th>Cadmium of soil</th>
<th>Essence percentage</th>
<th>Shoot dry matter</th>
<th>Shoot fresh matter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year(Y)</td>
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<td></td>
<td></td>
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<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Coefficient of variation</td>
<td>0.74</td>
<td>1</td>
<td>2.4</td>
<td>1</td>
<td>2.9</td>
<td>5.9</td>
<td>5.3</td>
<td>3.5</td>
<td>6.8</td>
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</tr>
</tbody>
</table>

Table 3: Results of correlation between characters in purslane plants that are affected by several heavy metals in second year.

<table>
<thead>
<tr>
<th>Characters</th>
<th>Root fresh matter</th>
<th>Root dry matter</th>
<th>Nickel of plant</th>
<th>Cadmium of plant</th>
<th>Cadmium of soil</th>
<th>Essence percentage</th>
<th>Nickel of soil</th>
<th>Cadmium of soil</th>
<th>Essence percentage</th>
<th>Shoot dry matter</th>
<th>Shoot fresh matter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root fresh matter</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Root dry matter</td>
<td>0.99</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nickel of plant</td>
<td>-0.21**</td>
<td>-0.21**</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nickel of soil</td>
<td>-0.22**</td>
<td>-0.22**</td>
<td>0.86</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cadmium of plant</td>
<td>-0.78**</td>
<td>-0.78</td>
<td>0.13**</td>
<td>0.04**</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Cadmium of soil</td>
<td>-0.73**</td>
<td>-0.71**</td>
<td>0.25**</td>
<td>0.25**</td>
<td>0.94</td>
<td>1</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Essence percentage</td>
<td>0.94</td>
<td>0.92</td>
<td>-0.21**</td>
<td>-0.21**</td>
<td>-0.75</td>
<td>-0.68</td>
<td>1</td>
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<tr>
<td>Shoot dry matter</td>
<td>0.91</td>
<td>0.9</td>
<td>-0.32**</td>
<td>-0.3**</td>
<td>-0.58</td>
<td>-0.51</td>
<td>0.93</td>
<td>1</td>
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<tr>
<td>Shoot fresh matter</td>
<td>0.84</td>
<td>0.82</td>
<td>-0.34**</td>
<td>-0.34**</td>
<td>-0.49**</td>
<td>-0.43**</td>
<td>0.89**</td>
<td>0.98**</td>
<td>1</td>
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</table>

Table 4: Results of correlation between characters in purslane plants that are affected by several heavy metals in second year.

<table>
<thead>
<tr>
<th>Characters</th>
<th>Root fresh matter</th>
<th>Root dry matter</th>
<th>Nickel of plant</th>
<th>Cadmium of plant</th>
<th>Cadmium of soil</th>
<th>Essence percentage</th>
<th>Nickel of soil</th>
<th>Cadmium of soil</th>
<th>Essence percentage</th>
<th>Shoot dry matter</th>
<th>Shoot fresh matter</th>
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<tbody>
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<td>Root fresh matter</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Root dry matter</td>
<td>0.9</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nickel of plant</td>
<td>-0.31**</td>
<td>-0.31**</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nickel of soil</td>
<td>-0.32**</td>
<td>-0.32**</td>
<td>0.96**</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Cadmium of plant</td>
<td>-0.88**</td>
<td>-0.75**</td>
<td>0.23**</td>
<td>0.12**</td>
<td>1</td>
<td></td>
<td></td>
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<tr>
<td>Cadmium of soil</td>
<td>-0.77**</td>
<td>-0.63</td>
<td>0.25**</td>
<td>0.25**</td>
<td>0.9</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Essence percentage</td>
<td>0.9</td>
<td>0.94</td>
<td>-0.22**</td>
<td>-0.1**</td>
<td>-0.77**</td>
<td>-0.78**</td>
<td>1</td>
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<tr>
<td>Shoot dry matter</td>
<td>0.92</td>
<td>0.9</td>
<td>-0.3**</td>
<td>-0.3**</td>
<td>-0.65</td>
<td>-0.55</td>
<td>0.94</td>
<td>1</td>
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<tr>
<td>Shoot fresh matter</td>
<td>0.88</td>
<td>0.8</td>
<td>-0.61**</td>
<td>-0.23**</td>
<td>-0.59</td>
<td>-0.45</td>
<td>0.86</td>
<td>0.9</td>
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</table>

ns, * and **: Non significant, significant at the 5% and 1% levels of probability, respectively.
Shoot dry matter in purslane plants that are affected by several heavy metals in first year.

Root dry matter in purslane plants that are affected by several heavy metals in first year.

Final Leaf Area Index in purslane plants that are affected by several heavy metals in first year.

Essence percentage in purslane plants that are affected by several heavy metals in first year.

Shoot dry matter in purslane plants that are affected by several heavy metals in second year.

Root dry matter in purslane plants that are affected by several heavy metals in second year.

Final Leaf Area Index in purslane plants that are affected by several heavy metals in second year.

Essence percentage in purslane plants that are affected by several heavy metals in second year.


