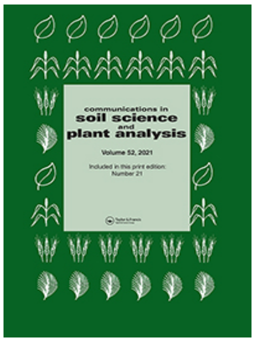

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Comparison of Common Vetch Plant Growth, Arsenic and Nutrients Uptakes in the Clean and Arsenic-Contaminated Soils

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ABSTRACT

Common vetch plant (*Vicia sativa* L.) growth under various arsenic (As) loads was investigated in the clean (1st year cultivation) and As-contaminated soils (2nd year cultivation). The total dry weight (DW) of common vetch gradually increased by the application of low concentration of As-contaminated irrigation waters (IWs) while the decrease of total DW to lower than the control plant at high As loads was observed. Compared with the control plant, the aboveground biomass yield loss was 7.6% and 61.6% in the 1st and 2nd cultivation years, respectively. The root dry (RDW) loss was about 36% in the 2nd cultivation year while the 1st year crop root yield loss was 56.5%. In the 1st cultivation year crops, the root As amount was about 1.8–1.9 fold higher than in the shoots while it reached to about 4–11 fold in the 2nd year crops. Although, common vetch might be defined as a highly tolerant plant to As in the clean soil, results demonstrated that the As tolerance of plant could decline when the usage of As-contaminated IWs for a prolonged time. Application of As-contaminated waters for a long time caused the changes of macro (N, P, Ca, Mg, and K) and microelements (Fe, Mn, Cu, Zn) transportation from the root to aboveground biomass.

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KEYWORDS
Arsenic; common vetch; irrigation waters; nutrients

Introduction

As, which is a toxic metalloid, can enter the environment from natural processes (such as weathering of As-rich minerals in Earth's and volcanic activity) and/or via anthropogenic sources (such as mining, burning of fossil fuels, excessive use of As–based fertilizers, and agrochemicals) (Abbas et al. 2018; Reed et al. 2015). As is not essential element for the growth of plants and it does not have a known metabolic function in the plant exposed at a low concentration of As. However, As is very hazardous for humans when it reaches to high concentrations through the food chain (Wu, Hong, and Yan 2015).

As is commonly encountered in the groundwater sources (Caporale et al. 2013). Groundwater is not only consumed as a drinking water, but also widely applied for the crops irrigation (Abedin et al. 2002). Use of As-contaminated groundwater for the plant irrigation may cause the increase of As level in the agricultural soils and crops (Dahal et al. 2008). Due to correlation between the concentration of As in the plant and waters was better than in the soil, As uptake of plants is related mainly to the As-contaminated irrigation water (IW) (Dahal et al. 2008).

The common vetch plant (*Vicia sativa* L.) is suitable for hay making (Berhane and Eik 2006) and is an important crop in the production of animal feed (Aslan 2018). It can easily grow around homesteads of smallholder farmers (Berhane and Eik 2006) and it is also an ideal plant for rotation or annual grain legume mixtures (Sabancı et al. 2016). The common vetch plant has a greater yield potential than

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the other vetch species and sown and harvested in a year. The seeds of the plant are locally available and the protein contents of the hay are fairly high (Berhane and Eik 2006).

Globally, the production area of vetches was approximately 573,769 ha, equating to a crop production yield of 926,982 tones/year (Huang et al. 2017). Vetch is also one of the most widespread (almost 22.4% of Turkey's cropland) annual legumes cultivated in Turkey and satisfied approximately 9% of the total animal green feed crops in 2017 (TSI 2018).

Effect of As on plant growth was widely studied and the most of study was carried out in the As-contaminated soil. However, there is lack of knowledge on the growth of common vetch plant under various As loads. The plant growth and transportation of nutrients and As from the root to the aboveground biomass by gradually increase of As load has not been studied until now.

In the present study, the effects of As-contaminated IW on the growth of common vetch plant cultivated in a greenhouse was investigated. Due to various concentrations of As were steadily added into the each pot with irrigation, the total applied As amount to the plants increased gradually throughout the experiment. Experimental studies were carried in the clean soils (1st cultivation year) and after harvesting of the plants, the As-contaminated soils in the first year were used for the plant cultivation in the 2nd year. The growth of plants under various As loads were determined considering the dry weight of each plant and compared with the control plant. Amounts of As, macro and microelements in the plant root and aboveground biomass (stem and leaf) were determined and compared for both cultivation years. Changes of the element transportation from the root to the aboveground biomass under various As loads in the common vetch plant was investigated in the clean and As-contaminated soils.

Materials and methods

Preparation of soil

Soils for the pot experiments were obtained from the upper layer of soil (0–30 cm) in the Campus Area of Sivas Cumhuriyet University and dried in the greenhouse without air conditioning. The air-dried soil was passed through from the 2 and 4 mm sieves. In the experimental studies, 10 kg soil in the pot was used as a growth medium after applying basic fertilization (100 mg/kg N ($Ca(NO_3)_2.4H_2O$), 100 mg/kg P and 125 mg/kg K (KH_2PO_4), and 2.5 mg/kg Fe (Fe–EDTA). Additionally, the plant micro nutrient mixing (w/w; B 1.5%, Fe 3.0%, Mn 3.0%, Zn 4.0%) was added into the soil (Aslan 2018). Some principal physicochemical properties of the soil are presented in Table 1.

Plant cultivation

The common vetch seeds (Kubilay-82) of 6 g were sown into each pot and the plants were grown in the greenhouse conditions. The pots were irrigated with equal volume of tap water during experimental study. The seeds amount was calculated according to the applied weight in the agricultural area of 90 kg/ha (Avcıoğlu, Hatipoğlu, and Karadağ 2009). Experimental study was conducted according to completely randomized design factorial with three replicates. Throughout the experiments the temperature of greenhouse was kept constant about 20–25°C, which was the optimal environmental condition for the common vetch plant growth.

Table 1. Physicochemical properties of soil.

Parameter	Value	Parameter	Value	Parameter	Value
Texture	Clay	P_2O_5 (kg/ha)	35.8	Fe (mg/kg)	3.22
pH	7.30	K_2O (kg/ha)	925	Zn (mg/kg)	0.45
As (mg/kg)	**0.012**	Org. Mat. (%)	1.3	Mn (mg/kg)	2.40
Salt (%)	0.029	Lime (%)	15.8	Cu (mg/kg)	1.19

The plants were irrigated with the tap water from sowing to germination. After germination, the plants were irrigated with various concentrations of As–contaminated tap water (0.00 (control)–0.50–1.00–1.50–2.00–2.50–3.00–4.00–6.00–8.00 mg As/L). The As contaminated waters were prepared by using 0.05 mol/L NaAsO2 (MerckIn order to maintain the water holding capacity of 70%, the pots weights were measured before each watering. The As loads applied to the each plant was calculated by Eq1.

$$As\ loads(mgpot) = amount\ of\ IWs(L) \times As\ concentrations\ in\ the\ IWs \quad (1)$$

The ranges of As concentrations in the IW were chosen to encompass the concentrations occurred in a groundwater of As affected areas in the world (Aslan 2018). The plants were irrigated with the total water volume of 24.8 and 15.8 L during the 1st and 2nd cultivation years, respectively.

The plant seeds were sowed into the clean soils and harvesting was carried out in the period when 10% of plants bloom. After harvesting of the plants, the root and soil samples from the pots were obtained. For the cultivation of common vetch plants in the 2nd year, As-contaminated soils in the 1st year were stored in the laboratory. After applying basic fertilization with the addition of macro- and micro-nutrients, the As-contaminated soil of 10 kg was used in the 2nd cultivation year. The cultivation, irrigation, and harvest of plants in the 2nd year were carried out as in the 1st year.

Sampling of root, aboveground biomass and soils

At the harvest, the aboveground biomass (shoot) and roots of common vetch were collected separately. In order to remove impurities on the plant samples, the plant tissues (root and shoot) were washed with the tap and deionized water. The tissues were dried in an oven (between 65°C and 75°C) for about 48 h to determine the dry weight of samples. The dried plant tissues were ground into powder by using grinding mill (HD–702 model, Simsekler Labortechnic) for the element analysis. The powdered tissue samples were cleaned with 0.1 N HCl solution. After harvesting of the plants, the soil samples were collected from the pots.

Element analysis

The powdered plant samples of 0.2 g were used in the element analyses. In order to determine the macro (P, K, Mg, N, and Ca) and micro elements (Cu, Mn, Fe, and Zn), the powdered samples were digested by using a microwave method (with HNO_3–H_2O_2 in the microwave oven) by using microwave oven (Milestone–ETHOS EASY).

After digestion of samples, P concentration was measured calorimetrically at 882 nm in the UV–spectrophotometer (Murphy and Riley 1962). Concentrations of K, Mg, Ca, Fe, Mn, Zn, and Cu in the samples were determined by the Atomic absorption spectrometer (Analytik Jena AG–contrAA 700 model) (Guzel et al. 1992; Kacar and Inal 2008). The total N concentration of samples was determined with the Kjeldahl method (Bremner 1965).

In order to determine the available As amount, 20 ml DTPA was added to 10 g of soil samples. After mixing about 2 h of the samples, the solutions were passed through from the blue band filter papers.

The total and available As concentration of plant and soil were measured with the hydride generation atomic absorption spectrophotometer (Analytik Jena AG–contrAA 700 model). The precision was calculated on three replicated for the digestion procedures.

Statistical analysis

The experimental data was subjected to ANOVA variance analysis with the use of SPSS23.0 (SPSS Inc, Chicago IL) for Windows packet for the statistical analysis. The differences between the applications were determined in a way that they were lower than 0.05 ($P < .05$) with Tukey's test.

Results and discussion

Common vetch growth under various As loads

Aboveground biomass growth

The total dry biomass of plant is considered as a critical parameter to evaluate the effects of As on the plant growth (Niazi et al. 2017). The shoot dry weight (SDW) of control plant, which is cultivated in the As free soil, was 46.0 g (Figure 1). When the As loads were elevated to 12.4 mg/pot (0.50 mg As/L) and 24.9 mg/pot (1.0 mg As/L), the SDW increased to 49.1 and 53.7 g, respectively. At low As dosages (≤2.0 mg As/L), although the SDW decreased gradually up to about 49.8 g, the SDW of As applied plants were higher than the control plant. It was observed that the plant growth is restricted when the application of high concentrations of As (>2.0 mg As/L). The SDW of plants with the applied As concentration of 8.0 mg/L decreased to about 42.5 g, which is lower than the control plant SDW.

In the As–contaminated soil (2nd year crops), significant SDW variations were not observed up to the dosage of 6.0 mg As/L. The control plant SDW was about 24 g and increased slightly to 24.9 g at the concentration of 1.0 mg As/L. It was observed that the SDW gradually decreased as the As load increased. The SDW sharply dropped to 16.3 and 9.2 g/pot when the As concentrations were elevated to 6.0 and 8.0 mg/L, respectively.

Comparison of the aboveground biomass yield in the clean and As-polluted soil

Although the plants were grown in the greenhouse conditions, due to the different harvest periods, the applied As loads to plants varied in the 1st and 2nd cultivation years. In the 2nd harvest year, the SDWs including control unit were lower than the 1st year's crops. Increase of SDW in the 2nd year's crops were negligible in the pots applied 0.5 and 1.0 mg As/L. However, it was observed that the SDW increase was about 8% up to the concentration of 2.0 mg As/L in the 1st cultivation year crops.

At the concentration of 8.0 mg/L, the As load was lower in the 2nd cultivation year (126.6 mg As/pot) than in the 1st year (198.8 mg As/pot). However, compared with the control plant, the aboveground biomass yield loss of 7.6% and 61.6% was observed in the 1st and 2nd cultivation years, respectively. When the SDW data are evaluated, it can be concluded that prolonged uses of As-contaminated waters for plant growth causes significant yield loss.

Presence of inorganic As forms at a low concentration in the growth medium and the exposure time of plant increase the protein content of plants (Duman et al. 2013). The plant growth stimulation at low As concentrations has been attributed to the total protein increase of plants (Finnegan and Chen 2012). Presence of As at a low concentration in the IWs, positively affected the growth of common vetch and SDW increase was observed in the experimental study. The positive effect of As on the

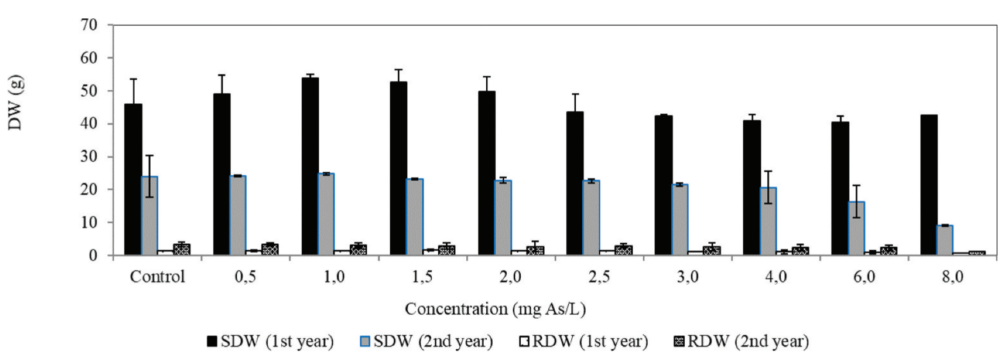

Figure 1. The dry weights of plant tissues at various As loads ($P < .05$).

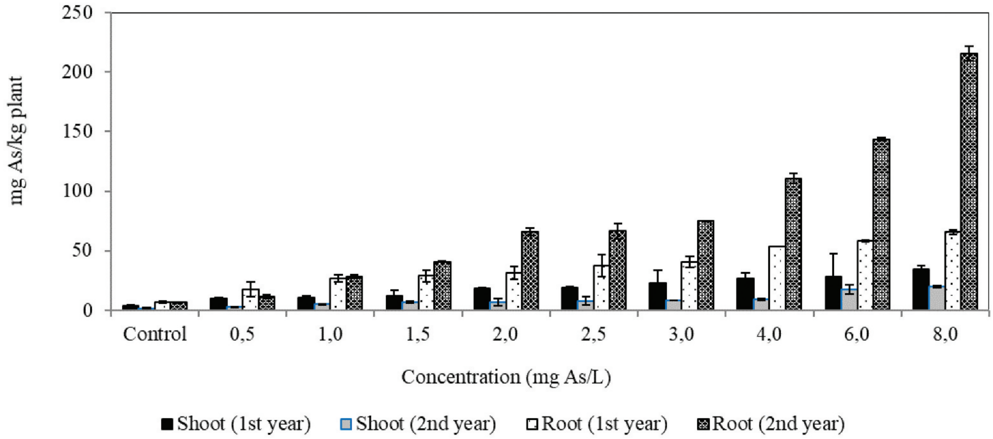

Figure 2. Arsenic accumulations in the root and shoots ($P < .05$).

growth and synthesis of plants was reported also for tomato (Miteva 2002), onion (Sushant and Ghosh 2010), red clower (Mascher et al. 2002), wheat (Li et al. 2007), *Baccharis dracunculifolia* (Gilberti et al. 2014), *P. vittata* (Singh and Ma 2006; Tu and Ma 2003), *Spartina alterniflora* (Carbonell et al. 1998).

As expected, in both cultivation years, when As concentrations elevated in the IW, As accumulation increased in the aboveground biomass. With the increase of As concentration from 0.5 to 8.0 mg/L in the IW, As content of aboveground biomass in the 1st and 2nd year's crops increased from 9.6 to 34.2 g/kg and from 2.7 to 19.9 g/kg, respectively (Figure 2).

Although the common vetch seeds were planted into the As-contaminated soils, because the As loads were higher in the 1st year than 2nd year, As accumulation in the aboveground biomass in 2nd year crops was lower than the plant grown in the clean soil.

In the 1st year, at the As load of 12.4 mg/pot (0.5 mg As/L), about 77% of the applied total As was kept in the aboveground biomass. The increase in As loads resulted in a decrease in the percentage of accumulation of total As applied in the shoots, with the As accumulation at the highest load falling to about 17% (198.8 mg As/pot, 8.0 mg As/L). In the As-contaminated soil, at the load of 7.9 mg As/pot (0.5 mg/L), about 34.5% of applied As to the plant was kept in the shoots, while the value was about 15.7% at the load of 126.6 mg As/pot (8.0 mg/L). At the dosage of 8.0 mg/L, the highest As accumulation in the As-contaminated soil was just about 58% of the 1st year. The upward transportation of As from the roots was restricted by the limitation of aboveground biomass growth at the high As loads. Similar results were observed for the studied As loads in this study.

Root growth

In the clean soil, the RDW of control plant was 1.5 g. Up to the As concentration of 1.5 mg/L in the IWs, the RDW increased to 1.7 g. When the As concentration was higher than 2.0 mg/L, the RDW dropped to the level lower than the dry weight of control plant. The lowest RDW of 0.6 g/pot was determined at the concentration of 8.0 mg As/L. Compared to the control plant RDW, the RDW loss at the highest As load was determined to be approximately 56.5%. (198.8 mg/pot) in the 1st cultivation year crop.

In the 2nd cultivation year, the highest RDW was determined in the control plant as 3.4 g. The increase in As concentration resulted in the decrease in RDWs, and the lowest value, 0.6 g, was observed at the As concentration of 8.0 mg/L.

Comparison of the root biomass yield in the clean and As-polluted soil

The RDWs of plants grown in the As-contaminated soil were determined to be higher than the RDWs of plants grown in the clean soil. At the concentration of 8.0 mg/L, the RDW loss in the 2nd year crop was about 36%, and this value was lower than the 1st year crop RDW loss of 56.5%. The decrease of RDWs was observed at high As concentrations (>2.0 mg/L) and all applied concentrations in the clean and As-contaminated soils, respectively. The decrease at high As loads could be attributed to the fact that the root of the plant is the first point of contact with As in the soil.

The root As amount steadily increased when the As concentration in IWs was elevated. At the As concentration of 0.5 mg/L (as a load of 12.4 mg/pot), the amount of As in the root of the plant grown in clean soil was determined as 17.2 mg/kg. The highest As accumulation in the root was 65.6 mg/kg at the As concentration of 8.0 mg/L. Although the As loads in the 1st year were higher than in the 2nd year, the As accumulation in roots were higher in the 2nd year than were 1st cultivation year crops (except for the concentration of 0.5 mg As/L). When the approximately applied equal As loads (62.1 and 63.3 mg As/pot) to the plants in the 1st and 2nd cultivation years were compared, the As accumulations in roots were determined to be 37.3 mg/kg (2.5 mg As/L) and 110.6 mg/kg (4.0 mg As/L), respectively. For both cultivation years, similar differences were also observed when the As concentration was higher than 0.5 mg/L.

Although the root As concentrations of control plants in the 1st and 2nd cultivation years were determined approximately equal (6.6 and 6.4 mg), the RDW in the 2nd year was about two times higher than the 1st cultivation year's crop. Differences of the As accumulation in roots between two cultivation years were generally 1.6–fold (at the concentration of 0.5 mg/L in the 2nd year) or more (at the concentration of 8.0 mg/L in the 2nd year).

Translocation of As in the tissues of common vetch

In the 1st cultivation year, about 66% (average value of all the applied As concentrations) of the total As was accumulated in the roots while the remaining was transferred to the shoots. The average As content of roots in the 2nd year crops reached to 87.3%, while about 12.7% of the total As in plant was accumulated in the shoots. In the clean soil, the root As amount was about 1.8–1.9 fold higher than in shoots while the level reached to about 4–11 fold in the 2nd year crops. The applied As loads to the plants were lower in the 2nd cultivation year than in the 1st year. However, results indicated that the root of common vetch plant accumulated more As in the contaminated-soil than in the clean soil. Accumulation of significant amounts of As, which is applied to the common vetch plant, in the root tissue may consist of a plant physiological mechanism that limits the transport of As to above-ground biomass (Stazi et al. 2016).

It was thought that both application of high As concentrations to the plants and usage of contaminated soil for the plant growth cause high level As accumulation in the roots of 2nd year' products. High amounts of As accumulations in the roots were also observed in some plants such as rice (Lei et al. 2013), ferns (Feng et al. 2015), and the mangrove species (*Kandelia obovata* and *Aegiceras corniculatum* L.) (Wu, Hong, and Yan 2015).

As amount in the aboveground biomass depends mainly on the root activity (Carbonell-Barrachina 1995) and upward As transport from the roots is often limited by the high toxicity of As to the radicular membranes (Wauchope 1983). Because the As non-hyperaccumulator plants tend to accumulate the most of As(III) in its root, low amount of As is transported to the aboveground biomass (Reed et al. 2013).

Although restriction on the root growth is not observed, high amount of As in the root may cause the transport functions could be damaged which results in the restriction of As transport from the roots to shoots might be occurred (Yanez et al. 2019). Consequently, because the most of applied As is kept in the root tissue of common vetch plant; the plant could be classified as an As non-hyperaccumulator.

Macro nutrients contents in the tissues

In the 1st cultivation year crops, significant differences of macro element amounts in the aboveground biomass were not observed with the addition of As in the IW (Figure 3). The highest N amount of 1.7% was determined in the control and 0.5 mg As/L applied pots. When the As dose was increased further, the shoot N level was determined to drop to lower levels than the control plant. While the shoots P level in the control pot was 0.17%, it elevated slightly to 0.23% at the As concentration of 1.0 mg/L and decreased gradually to 0.14% at the concentration of 2.5 mg As/L. Similar trends were also determined for the amount of K, Mg, and Ca in the shoots.

The concentrations of macro elements in shoots of common vetch plants grown in the clean and As–contaminated soils showed significant differences. In the 2nd year's crops, N, Mg, and Ca amounts in the shoots increased with increasing the As concentrations while the amounts of P and K decreased. For the applied As doses including control pots, the N concentrations in plants cultivated in the 2nd year were higher than the 1st year plants. The amounts of other macro elements (P, K, Mg, and Ca %) in the shoots of 2nd cultivation year's crops were lower than the 1st year's crops, except for the control plant.

The root N levels of plants growing under As stress were found to be higher than is 2.23%, which is the root N level of the 1st year control plant. Although significant P variations in the root were not observed, the control plant contained more K% and Mg % than the plants irrigated with As-contaminated IW. Considering the applied As doses, no meaningful results could be determined for the amounts of P, K, and Mg in root.

Compared to the control plant, the root K level was about 38% lower in the 8.0 mg as/L applied plant. Ca level increased from 2.2% to 2.6% when the As concentration was elevated to 1.5 mg/L. Further increase of the As concentration caused gradually decrease of the root Ca level and the lowest level of 1.3% was determined at the As concentration of 8.0 mg/L.

In the 2nd year crops, it was determined that N and Mg amounts in the root were higher than were the roots of control plant and 1st year crop too. Nitrogen was predominantly found in the roots of common vetch. Compared to the control plant, the concentration of N in plant tissues was slightly higher in the 1st year crops. On the other hand, concentrations of N in the tissue of shoot and root of the 2nd year crops were relatively higher (> %50) at the As concentration of 8.0 mg/L. Some competitive interactions in the absorption of the anionic forms of As and N taken up by the plants would be expected (Carbonell–Barrachina et al. 1997). However, opposite trend was observed in this study with the increase in As concentration and significant increase in the root N concentration was also determined.

It could be seen from the figure that the root of control plant contained more P compared with the plants exposed to As. However, significant differences of P amounts were not observed in the 1st and 2nd year's plants roots. The root Ca level of control plant was 0.38%. by the usage of As-contaminated waters, increase of Ca content in the root was observed and the level reached to the highest value of 0.62% at the concentration of 8.0 mg As/L. The amounts of roots macro elements such as N, Mg, and Ca increased with the increase of As concentration, while the amounts of P and K decreased in the 2nd cultivation year crops.

Results of the experimental study indicated that the changes of macro elements concentrations in the tissues under As stress. Usage of the As-contaminated waters for plant irrigation caused the elevation of Mg and Ca amounts in the plant tissues. While the concentrations of Mg, Ca, and P in the roots and shoots were about the same in the 1st year crops, the N concentration in roots was higher than the shoots in both cultivations years. In the 2nd cultivation year's crops, when the macro element concentrations of tissues were compared, results indicated that significantly higher levels of K and Ca in the shoots than roots. However, the macroelements N, P, and Mg were accumulated mostly in the roots rather than shoots.

Application of As-contaminated IWs in the plant growth caused the decrease of P and K amounts in the root and aboveground biomass. In particular, reductions in P concentration in the plant tissues

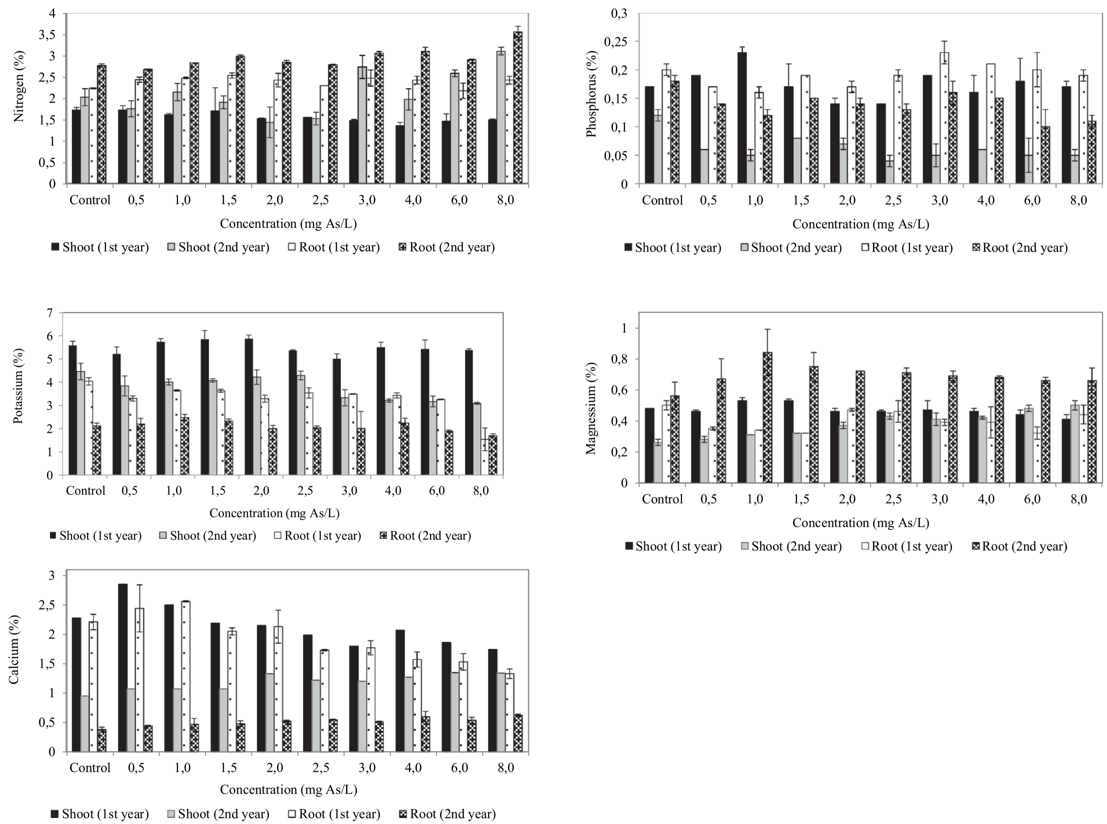

Figure 3. Changes of macro element concentrations in the plant tissues under As stress (%) ($P < .05$).

grown in the soils, which contains high amounts of As, are probably due to either As phytotoxicity or competitive uptakes (Gilberti et al. 2014). Changes in the total P concentration in the tissues of common vetch plants were negligible in the 1st year crop. However, in the As–contaminated soil, the root P concentration decreased with the increase of As concentration, this situation might be due to affinity of As compounds to phosphate channels (Shaibur et al. 2008).

In the most plants, P is concentrated in the aboveground biomass. However, distribution of P in the common vetch plant tissues shows unique trend with the higher P concentration in the root rather than the shoot whereas P concentration in the shoots decreased with the As exposure. When the concentration was higher than 4.0 mg As/L, the amount of P in root decreased about 40% compared to the control plant in the 2nd year. Changes in P concentrations were the most notable in the 2nd year crops tissues. In fact, for the applied As doses, about 50% reduction of P concentration in the shoots and roots were observed, with respect to the control plant. Although the root P concentration was not influenced significantly with increasing the As levels, the shoot P percent of control unit was about two times higher than the As applied pots in 2nd year. Reduction of P uptake by plants and increase of As concentration in the root is explained by the As damages to the root cell metabolism (Carbonell et al. 1998). Regarding the effect of As on the total P amount in the common vetch plant grown under different environmental conditions, similar results were reported (Hamid 2019; Ozturk 2018).

In both cultivation years, it was determined that the P/As ratios in the aboveground biomass were less than 1.0 for all applied As concentrations. Tu and Ma (2003) reported that the plant growth was retarded when the molar ratio of P/As in the fronds were lower than 1.0. However, under experimental conditions, the toxicity of As and growth retardation at the common vetch plant were not observed in this study. In contrast, higher biomass yields were achieved by promoting the plant growth at low As loads. Similar observation was reported on the DW increase at low As concentrations were reported (Hamid 2019; Ozturk 2018).

It could be concluded from the 1st year data that common vetch is equipped with an As detoxification mechanism including both P accumulation in the root and As transportation from the root to the aboveground biomass. However, in the 2nd year crops, compared to the control plant, less amount of P was accumulated in the plant tissues and also translocation of As from the root to the aboveground biomass was lower than was 1st year plants. At high As loads, it is thought that such a distribution pattern of P caused the decrease of the As detoxification capability of plant. Such a distribution pattern in the plant leads to a decrease in the plant's As detoxification capacity while eventually caused a lower biomass yield at high As loads. Singh and Ma (2006) explained this situation as the phytotoxicity effect of As on the plant.

Nutrient contents of common vetch plant found to be consistent with the reported in the literature (Gulumser et al. 2017; Hamid 2019; Ozturk 2018). P concentration of common vetch plant is reported between 0.09% and 1.98%. In the 1st year crops, the concentrations of total and tissue P in common vetch generally were about same as to that found in the control plant. Although P concentration in the plants exposed to As in the 2nd year were about half of the control plant, the plant total P amount was between 0.15 and 0.23 which were consistent with the literature.

In the 1st year plants, Ca levels of the roots and shoots were about 2.21% and 2.28%, respectively. By the increase of applied As loads, the root and shoot Ca levels gradually decreased, except for the concentrations of 0.5 and 1.0 mg/L. However, the levels of Ca were higher in the tissues than the control plant for the studied As concentrations in the 2nd year. Especially at high As loads, the As accumulation in both the root and shoot negatively influenced the Ca levels in the tissues. While As concentration in the plant tissues increased, the decrease of Ca concentration indicated that Ca had a limited role in the defense of common vetch plant against As toxicity. Similar suggestion has been reported by Tu and Ma (2004) and Carbonell et al. (1998).

Under the As stress of plants, although fluctuations of K level in the shoots were negligible, K amount in the root was about 38% of the control plant at the concentration of 8.0 mg As/L. The variations of K and Mg levels in plant tissues were same as Ca.

It was observed that the ratios of Ca/K in shoot and root depends on As concentration in the IW and soil. In both cultivation years, the Ca/K ratio in roots increased with increasing As concentration. In the 1st year, with the increase of As concentration from 0 to 8.0 mg/L, the Ca/K ratio dropped from about 0.41 to 0.32 in shoot. However, the shoot Ca/K ratio in the 2nd year crop's increased when the plants were exposed to As. Taking into account the concentration of elements in the control plant, the use of As-contaminated waters caused a slightly elevated Ca content in plant tissues (especially at the root) compared to Mg and K. As mentioned above, the DW of 2nd cultivation year crops was lower than the 1st year. It was assumed that due to the reduced growth yield of plants in the 2nd year, demand of macro elements were limited and contributed to the low uptake (Reed et al. 2015).

Plant micronutrient contents

Experimental results (Figure 4) indicated that the microelement concentrations of common vetch were within the range determined in the literature (Gulumser et al. 2017; Hamid 2019; Ozturk 2018). In the 1st year crops, at low concentrations of As, the microelements concentration of shoot were higher than the control plant while their amounts decreased with the addition of As. Although significant variations of microelement amount in common vetch were not observed for the As doses between 1.0 and 6.0 mg/L, the lowest microelement concentrations were determined at the As dosage of 8.0 mg/L. In the 2nd year crop, the highest concentration of microelements was observed in the shoots of control plant. When the crops of 1st and 2nd planting years were compared, microelement reduction in the 2nd year was higher than the 1st year at the concentration of 8.0 mg As/L.

Adding of 1.0 mg/L As into the IW, caused the increase of shoot Cu content from 17.7 mg/kg to about 22.5 mg/kg. Concentrations of Cu in the shoot decreased when the As concentration was higher than 1.0 mg/L. However, the shoot Cu contents of As applied plants were higher than the shoot of control plant, except for the As concentration of 8.0 mg/L.

The root Cu concentration of control plant was lower than the As applied plants. At the level of 2.5 mg As/L, the highest Cu concentration about 40 mg/kg was determined. The control plant root Mn level was about 70.8 mg/kg and at the concentration of 0.5 mg As/L, dropped drastically to about 43 mg/kg. However, up to the As concentration of 6.0 mg/L, increase of As content in the IW

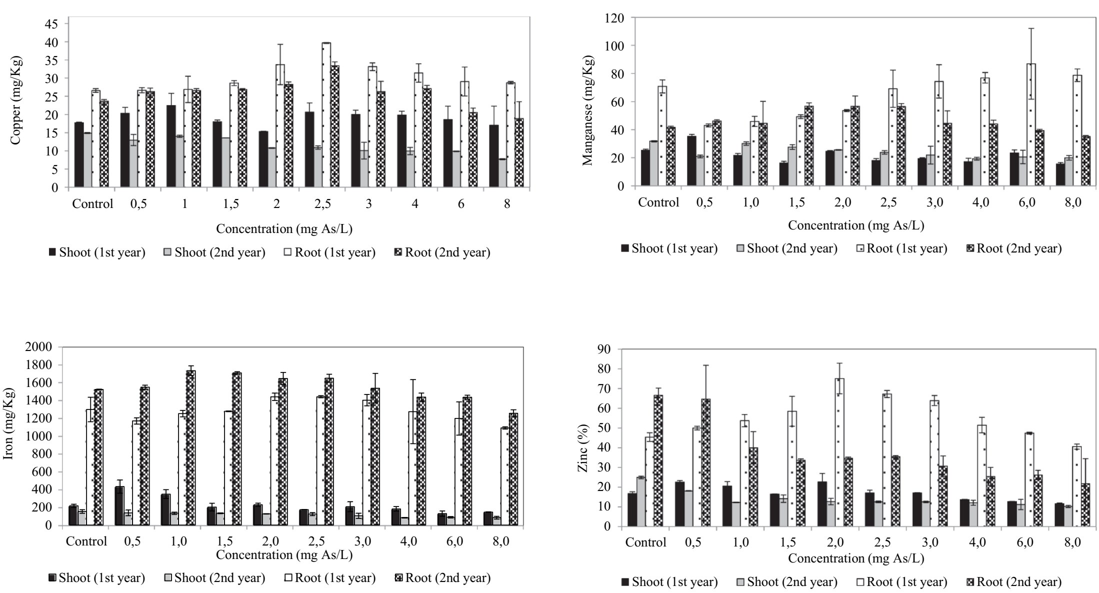

Figure 4. Changes of micro element concentrations in the plant tissues under As stress (mg/kg) ($P < .05$).

caused the elevation of Mn amount in the root increased gradually to about 86.7 mg/kg. The levels of Mn in root were higher than the level of control pot for the As concentrations between 3.0 and 8.0 mg/L. Significant variation of Fe amount in the root was not observed for the studied concentrations of As. Zn concentrations in plant root tissues were higher in the As-applied plants than in the control plant, except for the concentration of 8.0 mg As/ L. Zn concentration drastically decreased from about 66.6 to 21.7 mg/kg when the concentration of As increased from zero to 8.0 mg/L.

Except for Fe, other microelements Cu, Mn, and Zn concentrations in the roots of 1st year crops were higher than were 2nd year crops. Usage of As-contaminated waters for the growth of the common vetch plant changed the amounts of microelements in the common vetch plant tissues. It could be seen from the figure that both the plants which were grown in the 1st and 2nd cultivation years, the root microelements concentrations were higher than those the shoot. Further accumulation of microelements in the roots of common vetch and their internal transport from the root to aboveground biomass is explained by an exclusion strategy (Wang et al. 2009).

Soil As amounts

All of the available As applied to the pots was almost taken up by the common vetch plant. As can be expected, with the increase of As loads to the soils, available and total As amounts in the soil increased. In the clean soil, while the available As amount was lower than 0.1 mg/kg at the highest applied dosage of 8.0 mg As/L, the total As level was about 14.4 mg/kg. With the increase of As loads from 12.4 to 198.8 mg/pot, the total As holding capacity of soil decreased from 42.5% to 7.3%.

The available and total As levels of soil were between 0.05–0.21 mg/kg and 8.19–25.56, respectively in the 2nd cultivation year. Accumulation of As in the soil increased slightly to about 8.8% at the highest As loads in the 2nd year. Results indicated that the As holding capacity of soil declined with the increase of As load and the most of applied As by IWs was transferred to the plant tissues and low amount of As was accumulated in the soil structures. The level of As in soil increased due to the long-term application of As-contaminated IW in the plant growth. Although the As concentration changes in soil at As loads were negligible in the 1st cultivation year, in the 2nd year, the total As concentration of soil was significantly increased when the concentration of As in the IW was 6.0 mg/L or greater.

Conclusion

Long term uses of the As contaminated-waters in the plant growth caused a significant yield loss in the aboveground biomass. The RDW of plants grown in the As-contaminated soil achieved to about two fold of the 1st year RDW. Although a lower amount of As loads applied to the plants cultivated in the As contaminated-soils, As accumulation in the root of plants was higher than in the clean soils. It could be concluded that the translocation of As from the root to the aboveground biomass is restricted in the As contaminated soils.

The As loads affected the macro and micro elements uptakes and upward transport in the plant. Although experimental results indicated that the common vetch plant could be defined as highly tolerant to As in the clean soil, the As tolerance level of plant declined in the As–contaminated soil.

At high As exposure resulted in a significant reduction in biomass production but the plant survived, grew normally and the symptoms such as chlorosis, wilting and tissues necroses were not observed for the studied concentrations of As; this is demonstrating that the plant is an excluder with an innate capacity to tolerate As stress.

Long term uses of As-contaminated IW for the crop production might cause high As concentrations in the soils and elevated concentrations of As in the agricultural crops some of them used as animal fodder such as common vetch.

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