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Use of inductively coupled plasma optical emission spectrometry detection in determination of arsenic bioaccumulation in *Trifolium pratense* L. from contaminated soil

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Abstract. Increment of arsenic (As) in soil, a highly poisonous element, is a considerably important issue nowadays due to its danger of entry to the environmental cycles and food contamination. Bioaccumulation properties of many plants have been studied, although a very few reveal as a proper bioaccumulator plant for As. The Red clover, *Trifolium pratense* L. geographically widespread. The aim was conducted in order to determine the potential ability of this plant for cleaning up contaminated soil. Food grown on such lands usually contains heavy metals. Phytoremediation treatment of the soil prevents their entry into food, because it reduces the concentration of pollutants. Eighty-five Red clover one month old plants were grown in a nursery until transplant into the contaminated soil by inorganic As. Leaves and roots of 60 of plants sample were taken separately in every ten day during 60 days and analysed by ICP-OES while 25 samples were kept in different pH (8-4) in contaminated soil samples. Mean values of inorganic As(V) and As(III) in shoots and roots of plants were determined, and the statistical approaches were used for establishing the differences Bioaccumulation factor was calculated for As contents of plant parts for every 10 days. The soil arsenic level (19.09 mg/kg) higher than the global average (10.0 mg/kg), but within the maximum acceptable limit for agricultural soil (20.0 mg/kg) recommended by the European Union after 10 days. The lowest mean arsenic concentration was found after 40 days of cultivation of plant in pH=6 (1.01 mg/kg). It was observed that Red clover had suitable ability for phytoextraction method and soil recovery more Arsenic in pH<7 after 20-30 days of growth. The rate of As uptake by Red clover was significantly affected by the grown days after cultivated in contaminated treated ($p<0.05$) and pH of soil while up-taking in $pH\leq 6$ (p -value <0.05).

Key words: Phytoremediation, arsenic, contaminated soil, bioaccumulation, spectrometry.

1. Introduction

The cause of pollution of the soil on which food grows is anthropogenic activity (Voitiuk et al., 2014; Vambol S. et al., 2018, 2019; Kolesnyk et al., 2018; Sokolov et al., 2018). The introduction of phosphate fertilizers good reduces the mobility of heavy metals and their availability for absorption by plants. However, heavy metals accumulate in the soil and the soil becomes a secondary source of contamination of farmed foods. Among many pollutants, arsenic also enters the soil (Nguyen & Okolelova, 2013; Zariati et al., 2018). Weathering processes, biological and

geochemical reactions play a crucial role in the distribution of arsenic (Singh, 2006; Mohana & Pittman, 2007). Increased arsenic content in the soil as a very toxic element in nature (Mandal & Suzuki, 2002), whether as a result of natural phenomena or anthropogenic influences (Butcher, 2009; Zariati et al., 2018), is now a rather important problem due to its danger of entering food chains.

Through food produced on polluted soils of agricultural land heavy metals adversely affect human health. Chronic exposure to arsenic (when contaminated food is consumed) leads to a number of health problems, including respiratory, cardiovascular, renal, and skin diseases.

Solubility is the determining factor in the level of toxicity of arsenic compounds (Ng, 2005). Contaminated soils have more As(V) than As(III) concentrations (Butcher, 2009). Organic species of arsenic are generally considered less toxic than inorganic species for a wide range of organisms, including aquatic plants, animals, and people (Tamaki & Frankenberger, 1992).

Heavy metals remediation is by changing their oxidation level to another, or simply by transforming their complex (Marques et al., 2009). Arsenic has 4 different forms of oxidation (Smedley et al., 2002). Phytoremediation, which means using plants to deactivate the soil, is one of the new approaches to cleaning the soil from arsenic toxicity (Butcher, 2009; Bhattacharya, 2017). Phytoremediation consists of different methodologies and each of them have a different role in decontaminating arsenic contaminated sites. These methods are phytoextraction, phytostabilization, phytofiltration, rhizofiltration, phytoimmobilization, phytodegradation, rhizodegradation (Raskin et al., 1994; Ellen et al., 2005; Butcher, 2009; Ali et al., 2013).

Many plants, as accumulators of heavy metals, have been studied, although very few are disclosed as the correct bioaccumulator plant for arsenic. Progress in the study of plants requires a broad genetic basis. Knowledge of the genetic diversity of cultivated species and the diversity of their wild relatives is of great importance for the purposes of phytoremediation. Arsenate resistant plants can accumulate considerable levels of arsenic in their tissues, for example, 3470 $\mu\text{g/g}$ as in *Agrostis tenuis* and 560 $\mu\text{g/g}$ as in *Holcus lanatus* (Andrew, 2002; Favas, 2016). It was established experimentally that when growing maize, the transfer of arsenic from the roots to the shoots was significant. Therefore, As concentrations in leaves and grains exceeded the Swiss tolerance limits for feed and food crops (4 and 0.2 mg As kg^{-1} , respectively) (Gluz et al., 2005). It must therefore be assumed that arsenic-resistant plants either compartmentalise and/or transform arsenic to other less phytotoxic arsenic species in order to withstand high cellular arsenic burdens (Meharg, 1994).

The Red clover (*Trifolium pratense* L.) is biennial, but more often a perennial herbaceous plant. This plant is one of the most valuable forage grasses, which are valued for nitrogen fixation, which contributes to an increase in soil fertility. For this reason, it is used as a green dung culture.

At the same time, Red clover flowers and leaves are edible. Vitamin concentrates and essential oil are obtained from the leaves of Red clover. In medicine, red clover is used as an anti-inflammatory, antispasmodic and other means.

Therefore, as an element of the food chain, Red Clover (and the substances contained in this plant) can be a useful food product and at the same time have a negative effect if contaminated with heavy metals.

The positive factor is that Red clover geographically widespread. It grows throughout Europe (including Ukraine), it is widely distributed in North Africa (Algeria, Morocco, Tunisia), Western and Central Asia, different regions of Iran are suitable for its cultivation. This plant has the potential ability to phytoremediation. Thus, this study aims to determine the phytoremediation properties for phytoextraction of arsenic from contaminated arable land. To do this, the study solved the following tasks:

1. To determine the potential ability of Red clover to clean contaminated soil and their possible ability to phytoextraction arsenic to prevent the absorption of this toxic metal in the soil by agricultural crops.
2. To compare the phytoextraction ability of this plant at different stages of its growth.
3. To determine the transfer factors of the metal from the soil (TFS) of Red clover from contaminated soil samples.

2. Materials and Methods

2.1. Systematic review of open sources

To determine the features of Red clover and its growth sites the systematic review of open sources of information was conducted through Google Scholar (as a freely accessible web search engine of scholarly literature across an array of publishing formats and disciplines). Two queries with two key-words in the title and abstract were set for publications from 2016:

1. Red clover and grow
2. Red clover and arsenic.

It gave 6510 и 430 publications matching above criteria, what is 6940. The search results for each request were sorted by relevance and a 10 % cent sample was drawn for the first 100 publications from search results.

2.2. Experimental data

The contaminated soil by 20 µg/g DW Arsenic ((III)) and 15 µg/g DW Arsenic (V) , samples were filled in several plastic bags and carried to a laboratory at the Islamic Azad University, Nutrition and Food Sciences Research Center, Tehran-Iran. The Arsenic contaminated soil samples so collected were safely transported in clean self-sealing quart-size polyethylene freezer bag to the

Tehran laboratory. Eighty-five Red clover (*Trifolium pratense* L.) one month old plants were grown in a local nursery until transplant into the contaminated soil by inorganic arsenic (As) and hosted in 80 vases of 27 x 13 x 16 cm size and grown in the same conditions as those cultivated in vases. Each soil sample analyzed were a combination of 5 to 10 subsamples taken from the soil of each vase and mixes them together to obtain the final sample. Representative channel samples were taken from 0-25 cm depth from each vase.

Arsenic (III) and Arsenic (V) ion concentrations were determined as three replicates by Varian Vista Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES). A calibration curve was prepared to apply the linear relationship between absorbance and metal concentration in the concentration range being used. The intra-day and inter-day precision and accuracy of the method were determined under the optimal working conditions by triplicate measurements of known As concentrations. The first standard stock solutions had a 1.0 µg/L concentration of each metal and these were used for the preparation of aqueous standard solutions after appropriate dilution with 10 % nitric acid. The concentration ranges of the working solutions were 0.001-0.1 µg/L for all As states (Ziarati, 2012, 2014; Ziarati & Alimardan, 2015; Moghadam & Ziarati, 2016).

Leaves and roots of 60 numbers of plants sample were taken separately in every ten day in this study during 60 days and analyzed by ICP-OES while 25 samples were kept in different pH (8-4) in contaminated soil samples.

2. 3. Statistical Method

One-parametric Kruskal–Wallis/Mann–Whitney U tests were applied to compare differences between objects. Non-parametric multiple comparison test (Dunn's test) was performed to determine statistical significance of results at $\alpha = 0.05$. The GLM procedure was used for analysis of different time, pH and Arsenic state (3 and 5) with means separated by Duncan's multiple range test at $p < 0.05$. The CORR procedure was used for correlation analysis with means separated at $p < 0.05$.

3. Results and Discussion

3. 1. Characteristics of the studied plants and the growth area

The Red clover grows wildly in many parts of Iran in a vast range of habitats, mainly along the Alborz and Zagros Mountains. Although being economically important in many other countries (Thilakarathana et al., 2016; Broderick, 2018), information regarding the genetic diversity of this species in Iran is significantly lacking. The clover genus, *Trifolium* L., comprises about 255 species (Ellison et al., 2016) worldwide, from which about 48 species occur in Iran (Yousefi et al., 2018). The center of origin of the Red clover is thought to be the Mediterranean region (Southeastern Europe or Asia Minor) (Yousefi et al., 2018), from where the species distributed eastward through

Iran to Afghanistan and Pakistan (Vasileva & Ilieva, 2016; Yousefi et al., 2018). Red clover (*Trifolium pratense* L.) is economically second to alfalfa among the forage legumes, and is grown for hay, silage, forage and as a soil conditioner (Herbert et al., 2018). The Red clover is also an important component of temperate grasslands, which cover about eight percent of the global land area (McKenna et al., 2018).

3. 2. Determination the potential ability of Red clover to phytoextraction arsenic

Mean values of inorganic As (V) (arsenate) and As (III) in shoots and roots of plants were determined, and the statistical approaches were used for establishing the differences. Bioaccumulation factor (BAF) was calculated for As contents of plant parts for every 10 days of the study. The soil arsenic level (19.09 mg/kg) higher than the global average (10.0 mg/kg), but within the maximum acceptable limit for agricultural soil (20.0 mg/kg) recommended by the European Union after 10 days.

The diagrams show the change in the amount of As (III) and As (V) in the roots (Figs 1, 2), in the leaves (Figs 3, 4) and in the contaminated soil (Figs 5, 6).

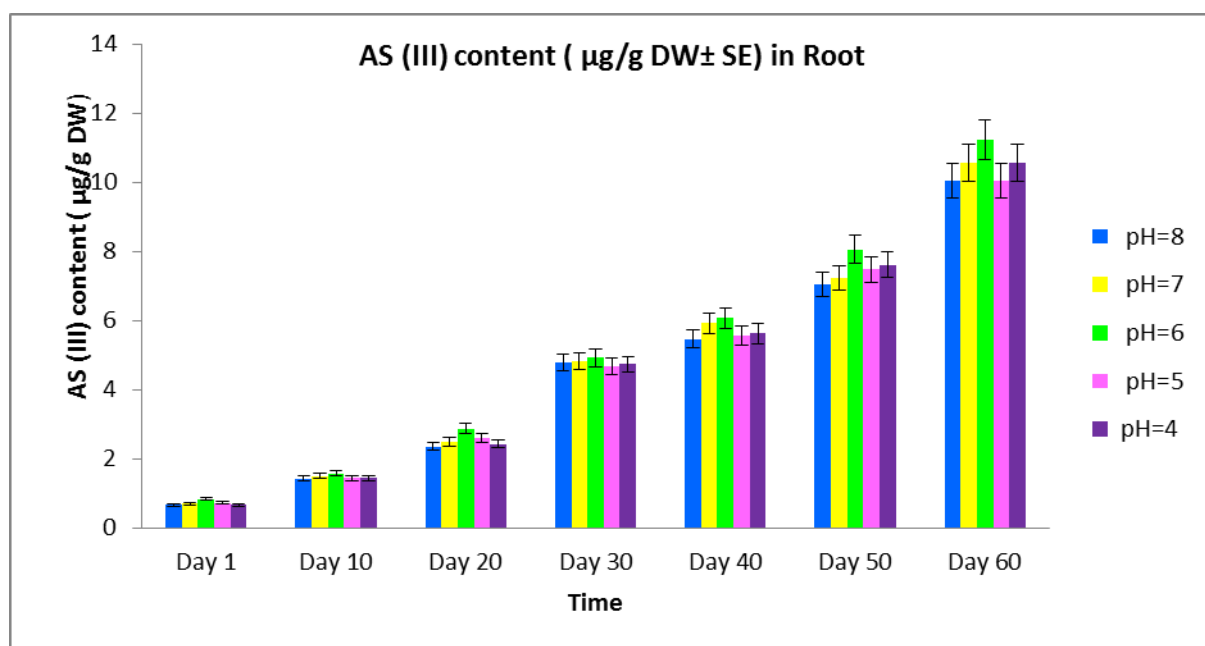


Figure 1. Content As (III) in root samples of the studied plants

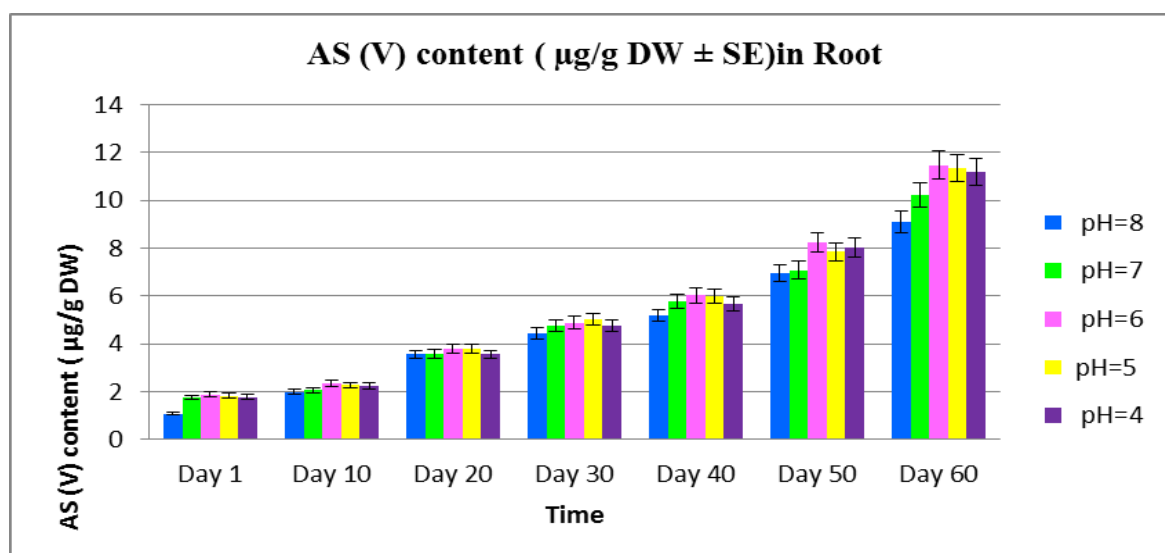


Figure 2. Content As (V) in root samples of the studied plants

Younger plants had more potential to uptake and concentrate Arsenic than older ones. Translocation factor in all conditions were higher than one which indicates that metal concentrations in shoots were higher than roots and the plant is suitable for phytoremediation.

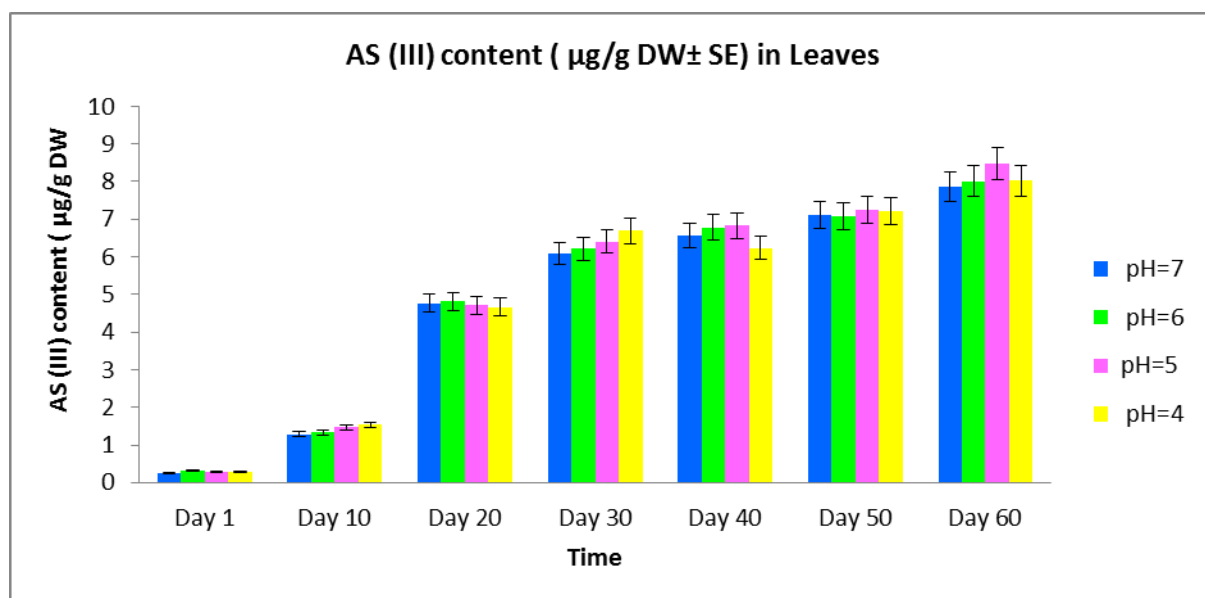


Figure 3. Content As (III) in leaves samples of the studied plants

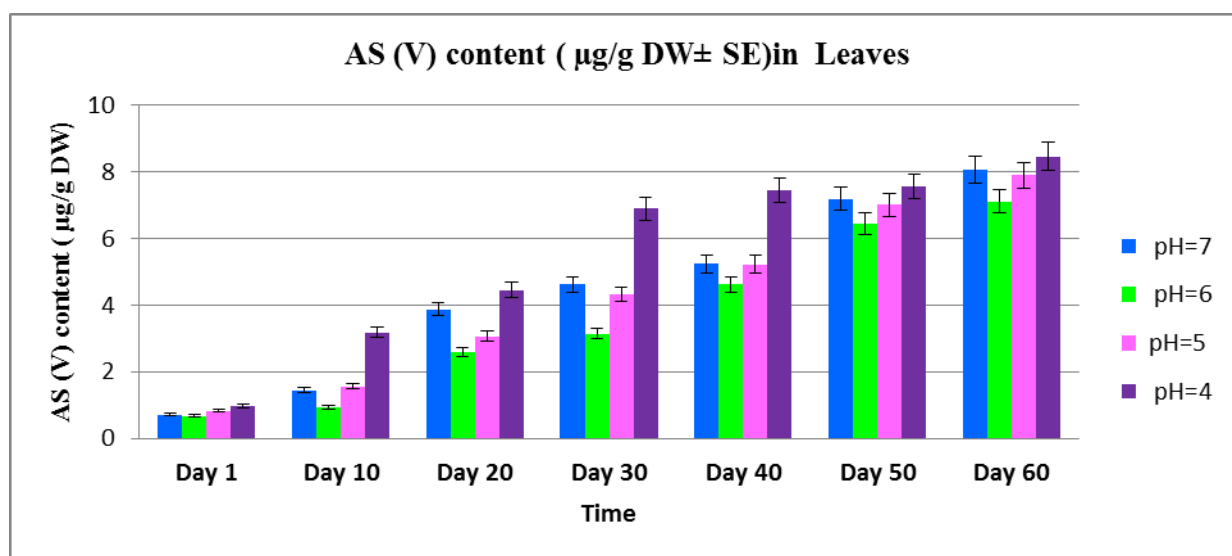


Figure 4. Content As (V) in leaves samples of the studied plants

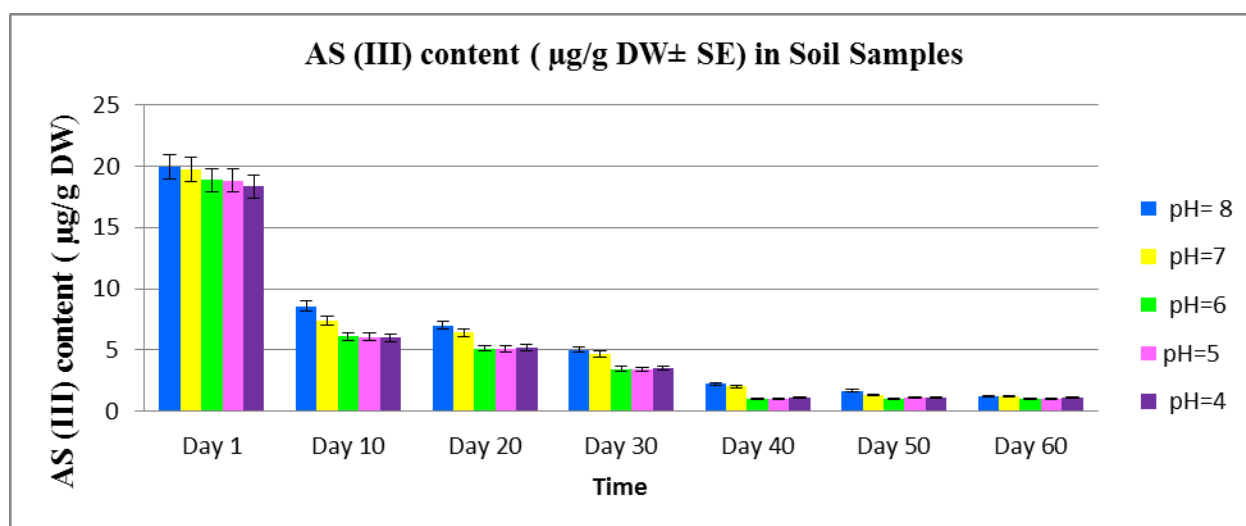


Figure 5. Content As (III) in soil samples

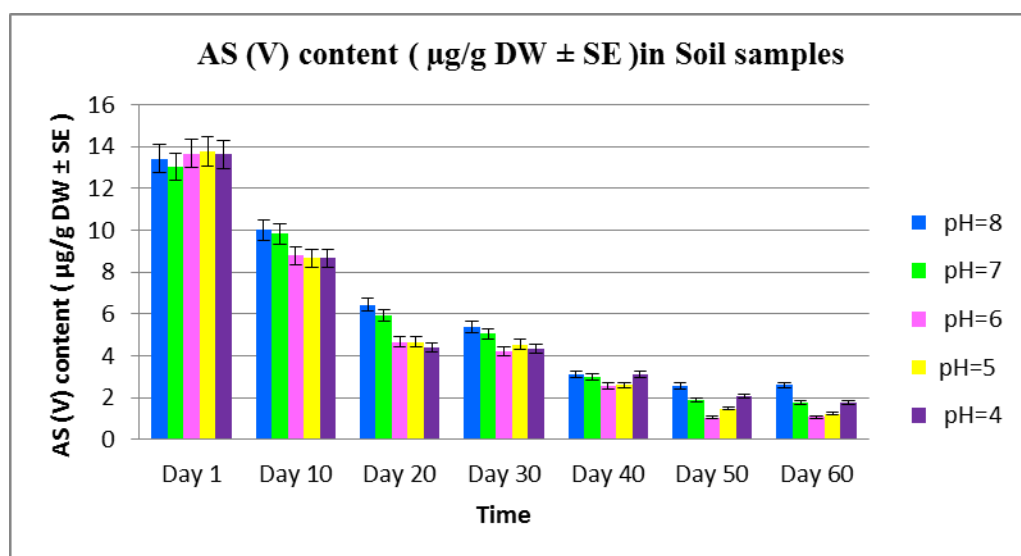


Figure 6. Content As (V) in soil samples

The lowest mean arsenic concentration was found after 40 days of cultivation of plant in pH=6 (1.01 mg/kg). The results of current research concluded that in the soil which consisted of Red clover had suitable ability for phytoremediation by phytoextraction method and transmitting more Arsenic in pH<7 after 20-30 days of growth of Red clover species. The rate of As uptake by *Trifolium pratense* L. was significantly affected by the grown days after cultivated in contaminated treated ($p < 0.05$) and pH of soil while up-taking in $pH \leq 6$ ($p\text{-value} < 0.05$).

4. Conclusion

The results of present study revealed adsorption capacity of As(V) and As(III) by Red clover (*Trifolium pratense* L.) was investigated in a batch system by considering the effects of various parameters like contact time, initial concentrations of contaminated studied elements and the pH of soil samples. The adsorption was time and metal dependent and the maximum adsorption was observed at As(III) contamination. Moreover, contact time of different pH in the contaminated soil showed significant ($p < 0.05$) and positive correlation with contents of As(III) and As(V) ($r = +95$ to $r = +92$) in the contaminated soil and Red clover grown time respectively. The amounts of As(III) and As(V) adsorbed increased significantly with increase contact time ($p < 0.005$). The results of this study revealed that Red clover residue can accumulate high level of As(III) and As(V) in a short time and their uptake rate by studied plant is significantly affected by their concentrations in the contaminated soil ($p < 0.05$). A contact time of 10 days by Red clover was found to be optimum and 91.2 % As(V), 95.6 % As(III) and only a very few amount of Arsenic was remained in soil, while a few amounts of these heavy metals being uptake by Red clover. Experimental results showed that low cost bio-sorbent was effective for the removal of pollutants from soil. The present

investigation shows that the Red clover after adsorbing Arsenic can be transformed as ash residue and could be effective in many industrial process and previous studied revealed that most arsenic used in industrial processes is used to produce antifungal wood preservatives and present study could be considered as very inexpensive adsorbent for the removal of contamination from soil.

This research conduct adsorption of heavy metals by agricultural waste and by-product and proved that this friendly method should gain more attention and research interest for the removal of heavy metals from contaminated soil due to its surface area, adsorption capacity and plenty abundant in nature must be followed seriously. It is obvious that many countries throughout the world such as Iran and other developing countries that are grappling with the issue of contaminated environments need a low cost and effective strategy. By determining the characteristics that influence environmental contamination by heavy metals especially Arsenic, Lead and Cadmium, scientists will be able to map high and low risks areas of contamination. Harvesting the certain plants to accumulate, up taken and translocations metals from roots to shoots can remove toxic and heavy metals from the soil and preventing vegetables, crops and other products to absorb them.

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