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Growth and biochemical responses of vetiver grass (*Vetiveria zizanioides*) to magnetized water and Pb

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ABSTRACT

An experiment was conducted to evaluate the phytoremediation efficiency, growth and biochemical responses of vetiver grass (*Vetiveria Zizanioides)* in response to irrigation with magnetized water [in 3 levels including magnetized water, semi-magnetized water and non-magnetized water] and Pb from the source of Pb-Nitrate salt [in 4 levels including 0, 5, 10 and 20 mg/L). This study was carried out as a factorial arrangement based on a completely randomized design with three replications in the central nursery of Green Area and Parks Organization Bandar Abbas Municipality, Bandar Abbas, Iran, during 2020–21. A magnetic water generating device called a magnetic ion stirrer with an intensity of 110 Tesla was used in this study. The results showed that irrigation with the magnetized water significantly increased the growth and yield of vetiver, and uptake and translocation factor of Pb to the shoots. Increase in the activities of enzymatic antioxidants affected by the magnetized water was observed which led to the production of reactive oxygen species (ROS) and activated the plant defense system. In general, despite high accumulation of Pb in plant tissues evident from accumulation of ROS, but still vetiver growth and yield were not significantly affected, indicating the phyto-toxic tolerance of vetivar against Pb accumulation. It is concluded that irrigation with the magnetized water through stimulating the antioxidants of vetiver can improve the growth, yield, uptake and translocation of Pb and therewith increases the phytoremediation efficiency of Vetiver.

Keywords: Lead (Pb), Magnetized water, Phytoremediation, Vetiver grass

Heavy metals are one of the main pollutants in the environment, and their toxicities has caused concern due to their adverse effects on public health (Mousavi *et al.* 2018a, Mousavi *et al.* 2018c, b, Moshiri *et al.* 2019). Lead (Pb) is a mineral pollutant for soil and a toxic element for plants and has a negative effect on plant growth and yield which anthropogenic activities including farming, manufacturing, transporting etc. cause Pb accumulation in the environment (Mousavi *et al.* 2010, Mousavi *et al.* 2013). In the recent years, numerous studies have been conducted on evaluating the efficiency of phytoremediation technique in the removal of potentially toxic metals from soil (Siyar *et al.* 2020, Gravand *et al.* 2021). One of the methods that have recently received a lot of attention is the use of magnetic field. Magnetization has been used to enhance seed germination, activate metabolism, biomass yield and increase plant resistance to heavy metals (Abdollahi *et al.* 2019).

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There have been many reports about increased defensive responses of plants affected by magnetic treatment (Razmjoo and Alinian 2017). The fact that magnetic treatment causes oxidative stress or increases the activity of plant defense enzymes, increases the concentration and longevity of free radicals, shows the correlation between magnetic therapy and its effects on plants (Sahebjamei *et al.* 2007). On the other hand, magnetic therapy can increase Pb uptake, which increases ROS production in plants and leads to oxidative stress such as hydrogen peroxide. Recent studies (Banejee *et al.* 2016, Pidatala *et al.* 2016) have solely reported on the phyto-extraction and phyto-stabilization effects of heavy metals uptake in Vetiver grass. Also, magnetized water has been studied by many researches however, to date, there has been little studies about the effects of magnetized water on efficiency of phytoremediation and biochemical responses of plants. Therefore, the objective of this study was to investigate the effects of magnetized water on biochemical responses of vetiver plant and its phytoremediation efficiency under Pb conditions.

MATERIALS AND METHODS

In order to study the effect of magnetic field on lead (Pb) uptake by vetiver grass, an experiment was conducted at the central nursery of Green Area and Parks Organization Bandar

Abbas Municipality (Latitude: during 27° 21' and Longitude: 56° 35' N), Bandar Abbas, Iran, during 2020–21. This pot experiment was conducted as a factorial arrangement based on a completely randomized design with three replications. The studied soil was sampled (surface layer, 0–30 cm) from an agricultural soil located in Baghu village, Bandar Abbas, Iran, and after air-drying, it was sieved (2 mm) to measure some physical [soil texture (Gee and Or 2002)] and chemical properties [*p*H (Klute 1986), Electrical Conductivity (EC) (Bremner J 1982), Organic Carbon (% OC) (Walkley and Black 1934), N (Bremner JM 1996), P (Olsen 1954) and available form of Fe, Mn, and Pb (Page 1982)]. The initial properties of the soil were as follows: Soil texture sandy loam, *p*H 7.52, EC 1.9 dS/m, organic matter 1.18%, total N 0.08%, P 6.5 mg/kg, K 30 mg/kg, Mn 6.1 mg/kg, Fe 4.3 mg/kg, and Pb not detectable. Each pot was filled with 10 kg of the soil.

The studied treatments included Pb in 4 levels (0, 5, 10 and 20 mg/l) from the source of Pb-Nitrate and irrigation water in 3 levels (magnetized water, semi-magnetized water and non-magnetized water). The Pb levels were added to the irrigation water. In the semi-magnetized water treatment, irrigation with magnetized water and non-magnetized water was done twice a week, so that once non-magnetized water was used and once magnetized water was used. The seeds of vetiver grass obtained from Seed and Plant Improvement Institute in Karaj, Iran were surface sterilized (Mousavi *et al.* 2018b, 2018c) and then germinated. Then, 3 vetiver plants with height of about 20 cm were transferred to the pots. According to the measured soil water content, the soil moisture was held near field capacity (FC) through irrigation with distilled water for one month until they were fully established. Based on the results of soil analysis, the nutrients requirements were added to the pots as fertigation. The duration of the pot experiment was 5 months. In order to magnetize the water, a magnetic water generating device (purchased from Fapan Company) called a magnetic ion stirrer with an intensity of 110 Tesla was used in this study. The device consists of a non-magnetic tube (steel) on which a number of magnets were placed in a ring on it in such a way that produces a dense and variable (i.e., sinusoidal) magnetic field.

At the end of the experiment, the concentration of available Pb in the soil (Page 1982) and roots and shoots (Abdolmaleki *et al.* 2007) was measured. Also, activity of Superoxide Dismutase (SOD) (Ghanati Faezeh *et al.* 2007), Catalase (CAT) and Peroxidase (POD) (Sahebjamei *et al.* 2007) was measured. Some phytoremediation indexes including translocation factor (TF), bioaccumulation factor (BF) and uptake index of Pb in root and shoot (UI) were calculated (Li *et al.* 2007). Finally, in order to statistical analysis of the data, SAS 9.1 software was used, and in order to compare the means, Duncan's multiple-range test (DMRT) (P<0.05) was used and graphs were drawn using Excel software.

RESULTS AND DISCUSSION

The results of variance analysis showed that the studied treatments significantly affected growth and biochemical responses of the plant. Also, interaction effects of the Pb levels and the magnetized water were significant on the studied traits (with the exception of shoot dry weight and translocation factor (TF). Among the enzymatic antioxidants, simple and interaction effects of the treatments significantly affected SOD and POD activities, while CAT activity was only affected by the Pb levels.

Dry biomass yield of the roots and shoots: Interaction effects of the treatments on dry biomass yield of root showed that with increasing the Pb level from 0 to 20 mg/L, its amount decreased, as well. So that the highest root biomass yield was recorded in absence of Pb (Fig 1a). Increasing of the Pb levels had a negative effect on the shoot biomass yield, so that the lowest shoot biomass yield, with about 18.73% decrease compared to the control treatment (i.e., without any Pb contaminations) (Table 1) was measured in the highest level of Pb concentration. Since the roots were damaged by Pb toxicity, increasing the Pb levels reduces the dry weight of shoots and roots (Mousavi *et al.* 2018a, Mousavi *et al.* 2018c, Moshiri *et al.* 2019). Razmjoo and Alinian (2017) also reported that changes in the chemical properties caused by irrigation with magnetized water resulted in increase in biological activity in plants, which influenced plant growth.

Pb accumulation, uptake and translocation: Efficiency of the magnetized water in reducing the accumulation of Pb in the soil was greater in the higher concentrations of Pb compared with the lower concentrations (Fig 1b). Also, the efficiency of irrigation with the magnetized water in increasing the accumulation of Pb in the root was increased with increasing the Pb levels, so that the highest accumulation of Pb in the root was measured in the Pb level of 20 mg/L under irrigation with the magnetized water and had a significant difference with other treatments (Fig 1d). This status was observed for accumulation of Pb in the shoot under different treatments (Fig 1c).

The translocation factor (TF) was significantly increased affected by the magnetized water. So that the highest TF of Pb, with about 38.3% increase compared to the control treatment (W3), was recorded in the magnetized water

Table 1 Some growth and biochemical responses of vetiver plant affected by different Pb concentration

Pb concentration (mg/L)	Shoot dry weight (g pot-1)	Catalase (nmol/min/ml)	TF
0	77.4 ± 2.28^{a}	0.088 ± 0.0014^{c}	0.00 ± 0.00^{c}
5	70.9 ± 1.19^{a}	0.092 ± 0.0022^{bc}	0.542 ± 0.052^{b}
10	68.1 ± 3.06^{b}	0.097 ± 0.0019^{b}	0.604 ± 0.023^{ab}
20	62.9 ± 2.08^{b}	0.110 ± 0.0032^{a}	0.619 ± 0.040^{a}

Data presented are the mean ± SE, significant differences at P<0.05 have been indicated with different letters.

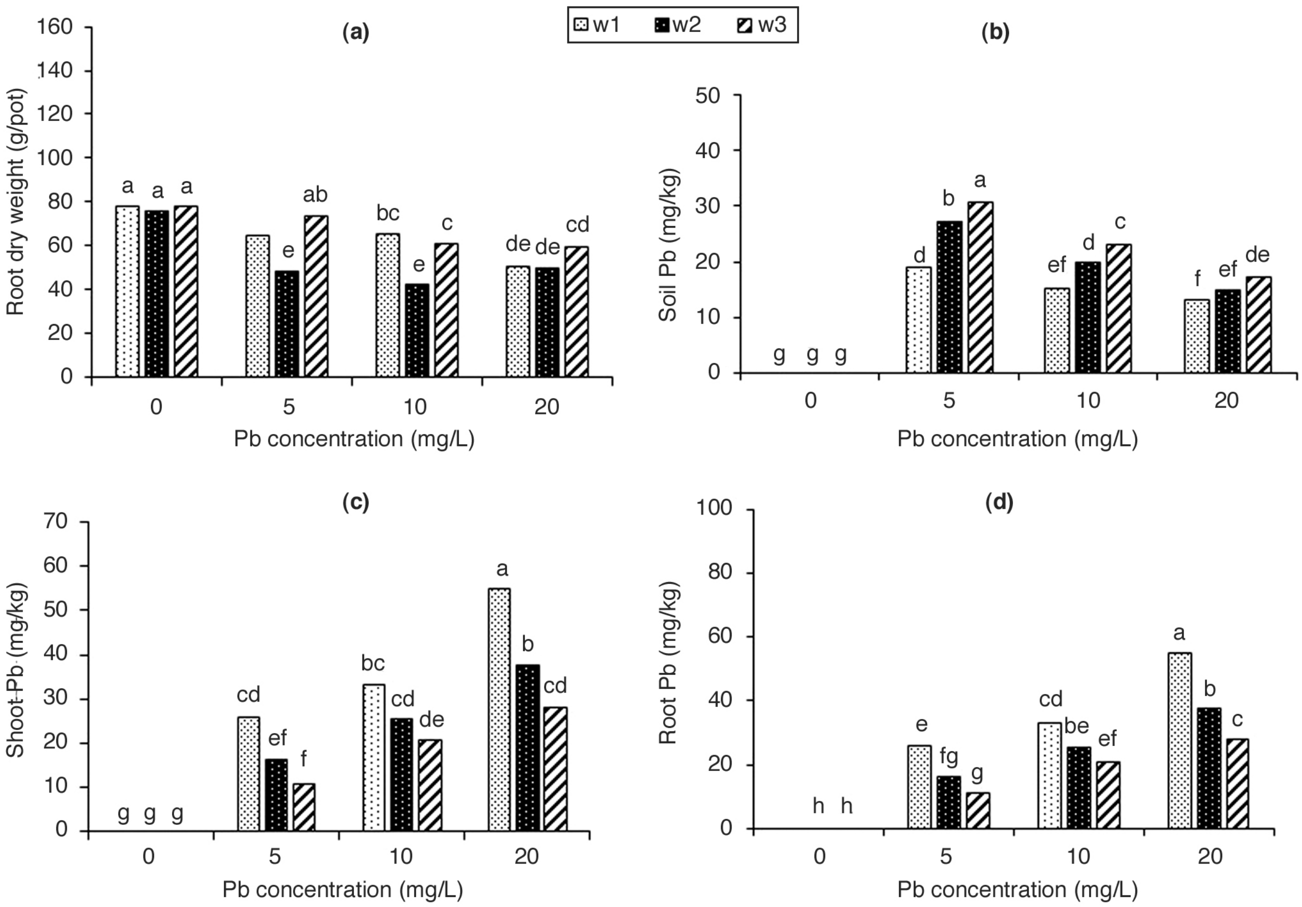

Fig 1 Effect of different treatments on a) root dry weight; b) Pb content of soil; c) Pb content of shoot; and d) Pb content of root. Significant differences at P<0.05 have been indicated with differences letters in each chart.

treatment. Uptake index of Pb by the root and shoot was significantly increased with increasing the Pb levels in different irrigation treatments (Table 2). The highest uptake index of Pb by the root and shoot was recorded in the Pb level of 20 mg/L when it was irrigated with the magnetized water and it was respectively about 19.43% and 79.35% more than the non-magnetized water (Table 2). With increasing the Pb levels and also with magnetizing the water, BF was increased, as well (Table 2). Regardless of the Pb level of 0, the lowest BF was measured in the Pb level of 5 mg/L under the non-magnetized water and it was about 6.13 times less than the highest BF which happened in the Pb level of 20 mg/L under the magnetized water (Table 2).

The measured BF and TF showed that under irrigation with the magnetized water, vetiver has a tendency to re-distribute further Pb into root to protect the sensitive organs. Under different irrigation treatments, BF, TF and uptake of Pb by vetiver was significantly increased with increasing the Pb levels. On the other hand, the magnetized water, increased concentrations, uptake, BF, and TF values, implying that external magnetic fields would push further Pb to fewer sensitive organs. According to these findings, *vetiver* may be considered as a hyperaccumulator plant (BF>1). Although there have been few studies on the effect of magnetic fields on capability of plants in metal uptake, there are a few studies that are important. According to Ali *et al.* (2013), the magnetic field can activate the membrane carriers, which can extract the heavy metals that are relatively mobile.

Enzymatic antioxidant activities: The highest activity of CAT was recorded in the Pb level of 20 mg/L which was about 25% more than the control treatment and has a significant difference with other treatments (Table 1). Irrigation with the magnetized water, especially in presence of the Pb levels, has a positive effect on the activity of POD (Fig 2a) and in absence of Pb, the magnetized water has no significant effect on POD. A similar status was observed for SOD. With increasing the Pb levels and also with using the magnetized water, its activity increased, as well. The highest activity of SOD was recorded in the Pb level of 20 mg/L when the magnetized water was used (Fig 2b). In absence of Pb, activity of SOD in the magnetized water was about 25% more than the non-magnetized water (Fig 2b).

SOD and POD are two essential antioxidant protective enzymes that can protect plants from the adverse effects of ROS caused by various stress conditions (Kataria *et al.* 2017). Increased activity of SOD can enhance toxicity of

Table 2 Interaction effects of the studied treatments on Root UI, Shoot UI and BF in vetiver plant

Magnetized water	Root Uptake Index (Root UI)			
	Pb concentration (mg/L)			
	0	5	10	20
W1	0 ± 0cA	2.45 0 ± 0.243bA	3.28 0 ± 0.255aA	3.75 ± 0.111aA
W2	0 ± 0cA	1.48 0 ± 0.208bB	1.83 0 ± 0.148bB	3.26 ± 0.067aAB
W3	0 ± 0cA	2.10 0 ± 0.249bA	2.24 0 ± 0.0.024bB	3.14 ± 0.407aB
Magnetized water	**Shoot Uptake Index (Shoot UI)**			
	Pb concentration (mg/L)			
	0	5	10	20
W1	0 ± 0dA	1.74 ± 0.144bA	2.00 ± 0.262bA	3.30 ± 0.173aA
W2	0 ± 0dA	1.14 ± 0.028cB	1.81 ± 0.040bAB	2.42 ± 0.070aB
W3	0 ± 0dA	0.80 ± 0.063cC	1.5 ± 0.080bB	1.84 ± 0.070aC
Magnetized water	**Bioaccumulation Factor (BF)**			
	Pb concentration (mg/L)			
	0	5	10	20
W1	0 ± 0dA	2.01 ± 0.275cA	3.28 ± 0.297 bA	5.64 ± 0.079aA
W2	0 ± 0dA	1.12 ± 0.119cB	2.19 ± 0.084bB	4.36 ± 0.102aB
W3	0 ± 0dA	0.92 ± 0.065cB	1.62 ± 0.178bC	3.03 ± 0.146aC

Data presented are the mean ± SE. For each column (lowercase letters at each Pb concentration) and row (capital letter at each irrigation water treatment) for root or shoot dry weight, significant differences at P< 0.05 have been indicated with different letters. W1, magnetized water; W2, semi-magnetized water; W3, non-magnetized water.

cell by reactive oxygen species (ROS) (Podleśny *et al.* 2021). Magnetic treatment increases the activity of SOD and POD and also significantly leads to eliminate the oxygen species, thus preserving photosynthetic membranes. Results showed that the Pb levels significantly increased the enzymatic antioxidant activity in the irrigation treatments. The increase in SOD activity is due to the plant's protection mechanism being protected from oxidative damage and stress. SOD is important in antioxidant systems because it eliminates superoxides (Gill and Tuteja 2010). An increase in the activity of POD was observed in vetiver under Pb stress in this study. This enzyme limits ROS production and reduces plant oxidative damage (QAMER *et al.* 2021). In this study, the activity of CAT significantly increased after exposure to Pb, which is corroborated by the results of Malar *et al.* (2016).

Antioxidant enzymes detoxify ROS through a series of synergistic reactions (Dvorak *et al.* 2020). However, it is important to note that one of the new aspects of our research is to determine the effect of magnetized water on enzymatic antioxidant activity and phytoremediation efficiency of Pb by vetiver plant. The magnetic treatment led to an increase in Pb concentration and accumulation and subsequent activity of enzymatic antioxidants in the shoots. As a result, increasing Pb uptake and antioxidant content in vetiver plants by applying magnetic treatment is beneficial for phytoremediation.

The results showed that irrigation with the magnetized water under different Pb levels significantly affected the growth and biochemical responses of vetiver. Most of the enzymatic antioxidants in vetiver were significantly increased affected by the Pb levels and irrigation with the magnetized water. Efficiency of vetiver on phytoremediation of Pb was significantly increased affected by the magnetized water. However, with increasing the Pb levels from 0 to 20 mg/L the potential of magnetized water was decreased. In general, it is concluded that irrigation with the magnetized water through stimulating the antioxidants in vetiver can

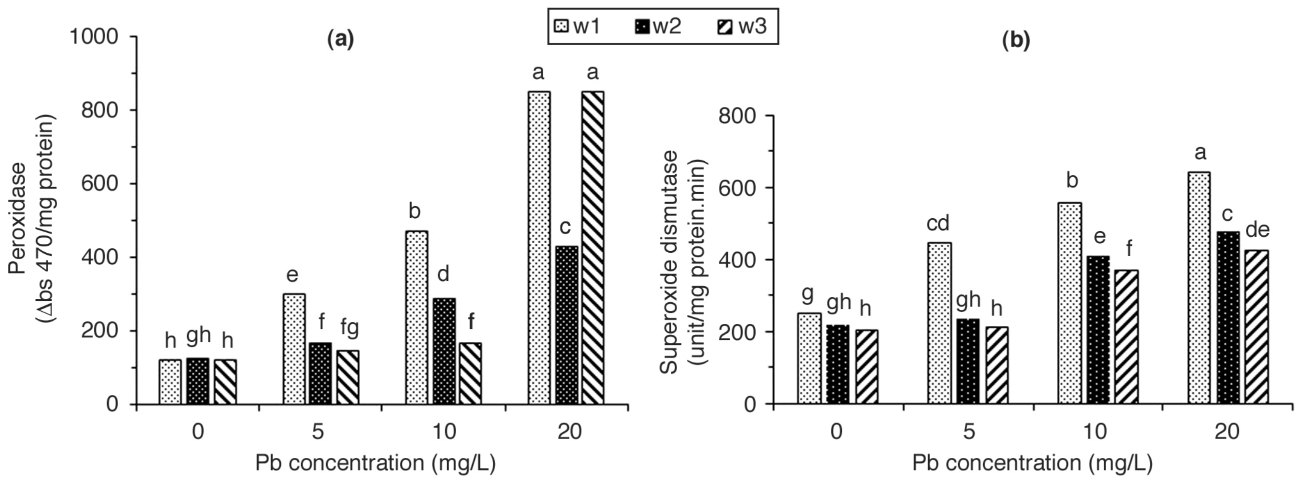

Fig 2 Activity of Peroxidase (a) and Superoxide dismutase (b) affected by the treatments. Significant differences at P<0.05 have been indicated with differences letters in each chart.

improve the growth, yield, uptake and translocation of Pb and therewith, increases the phytoremediation efficiency of vetiver.

REFERENCES

Abdollahi F, Amiri H, Niknam V, Ghanati F and Mahdigholi K. 2019. Effects of static magnetic fields on the antioxidant system of almond seeds. *Russian Journal of Plant Physiology* **66**(2): 299–307.

Abdolmaleki P, Ghanati F, Sahebjamei H and Sarvestani A S. 2007. Peroxidase activity, lignification and promotion of cell death in tobacco cells exposed to static magnetic field. *The Environmentalist* **27**(4): 435–40.

Ali H, Khan E and Sajad M A. 2013. Phytoremediation of heavy metals—concepts and applications. *Chemosphere* **91**(7): 869–81.

Bremner J. 1982. Total nitrogen. *Methods of Soil Analysis*. American Society of Agronomy monograph on Methods of Soil Analysis **10**(2): 594–624.

Bremner J M. 1996. Nitrogen-total. *Methods of Soil Analysis: Part 3. Chemical methods* **5**: 085-1121.

Dvorak P, Krasylenko Y, Zeiner A, Samaj J and Takac T. 2020. Signaling toward ROS-scavenging enzymes in plants. *Frontiers in Plant Science* **11**: 2178.

Gee G W and Or D. 2002. 2.4 Particle-size analysis. *Methods of Soil Analysis: Part 4 physical methods* **5**: 255–93.

Ghanati F, Abdolmaleki P, Vaezzadeh M, Rajabbeigi E and Yazdani M. 2007. Application of magnetic field and iron in order to change medicinal products of Ocimum basilicum. *The Environmentalist* **27**(4): 429–34.

Gill S S and Tuteja N. 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry* **48**(12): 909–30.

Gravand F, Rahnavard A and Pour G M. 2021. Investigation of Vetiver Grass Capability in Phytoremediation of Contaminated Soils with Heavy Metals (Pb, Cd, Mn, and Ni). *Soil and Sediment Contamination: An International Journal* **30**(2): 163–86.

Kataria S, Baghel L and Guruprasad K. 2017. Alleviation of adverse effects of ambient UV stress on growth and some potential physiological attributes in soybean (Glycine max) by seed pre-treatment with static magnetic field. *Journal of Plant Growth Regulation* **36**(3): 550–65.

Klute A. 1986. Methods of soil analysis-Part I. *Physical and Mineralogical Methods*. Soil Science Society of America, Inc Publisher, Madison, WI.

Li B, Zhou D, Cang L, Zhang H, Fan X and Qin S. 2007. Soil micronutrient availability to crops as affected by long-term inorganic and organic fertilizer applications. *Soil and Tillage Research* **96**(1-2): 166–73.

Malar S, Vikram S S, Favas P J and Perumal V. 2016. Lead heavy metal toxicity induced changes on growth and antioxidative enzymes level in water hyacinths [Eichhornia crassipes (Mart.)]. *Botanical studies* **55**(1): 1–11.

Moshiri F, Ebrahimi H, Ardakani M R, Rejali F and Mousavi S M. 2019. Biogeochemical distribution of Pb and Zn forms in two calcareous soils affected by mycorrhizal symbiosis and Alfalfa rhizosphere. *Ecotoxicology and environmental safety* **179**: 241–48.

Mousavi S M, Bahmanyar M A and Pirdashti H. 2013. Phytoavailability of some micronutrients (Zn and Cu), heavy metals (Pb, Cd), and yield of rice affected by sewage sludge perennial application. *Communications in soil science and plant analysis* **44**(22): 3246–58.

Mousavi S M, Bahmanyar M A, Pirdashti H and Gilani S S. 2010. Trace metals distribution and uptake in soil and rice grown on a 3-year vermicompost amended soil. *African Journal of Biotechnology* **9**(25): 3780–85.

Mousavi S M, Moshiri F and Moradi S. 2018a. Mobility of heavy metals in sandy soil after application of composts produced from maize straw, sewage sludge and biochar: Discussion of Gondek *et al.* (2018). *Journal of environmental management* **222**: 132–34.

Mousavi S M, Motesharezadeh B, Hosseini H M, Alikhani H and Zolfaghari A A. 2018b. Geochemical fractions and phytoavailability of zinc in a contaminated calcareous soil affected by biotic and abiotic amendments. *Environmental geochemistry and health* **40**(4): 1221–35.

Mousavi S M, Motesharezadeh B, Hosseini H M, Alikhani H and Zolfaghari A A. 2018c. Root-induced changes of Zn and Pb dynamics in the rhizosphere of sunflower with different plant growth promoting treatments in a heavily contaminated soil. *Ecotoxicology and environmental safety* **147**: 206–16.

Olsen S R. 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. Publisher: US Department of Agriculture. 19 pages.

Page A L. 1982. *Methods of Soil Analysis, Part 2-Chemical and Microbiological Properties.* Soil Science Society of America.

Podlesny J, Podlesna A, Gładyszewska B and Bojarszczuk J. 2021. Effect of pre-sowing magnetic field treatment on enzymes and phytohormones in Pea (*Pisum sativum* L.) seeds and seedlings. *Agronomy* **11**(3): 494.

Qamer Z, Chaudhary M T, Xiongming D, Hinze L and Azhar M T. 2021. Review of oxidative stress and antioxidative defense mechanisms in *Gossypium hirsutum* L. in response to extreme abiotic conditions. *Journal of Cotton Research* **4**(1): 1–9.

Razmjoo J and Alinian S. 2017. Influence of magnetopriming on germination, growth, physiology, oil and essential contents of cumin (*Cuminum cyminum* L.). *Electromagnetic biology and medicine* **36**(4): 325–29.

Sahebjamei H, Abdolmaleki P and Ghanati F. 2007. Effects of magnetic field on the antioxidant enzyme activities of suspension-cultured tobacco cells. *Bioelectromagnetics: Journal of the Bioelectromagnetics Society, The Society for Physical Regulation in Biology and Medicine, The European Bioelectromagnetics Association* **28**(1): 42–47.

Siyar R, Ardejani F D, Farahbakhsh M, Norouzi P, Yavarzadeh M and Maghsoudy S. 2020. Potential of Vetiver grass for the phytoremediation of a real multi-contaminated soil, assisted by electrokinetic. *Chemosphere* **246**: 125802.

Walkley A and Black I A. 1934. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil science* **37**(1): 29–38.