



## Poultry Manure Biochar Reduces Arsenic Induced Oxidative Stress and Arsenic Levels in Rice Plants

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Geliş Tarihi:26.12.2016; Kabul Tarihi:03.03.2017

**Abstract:** The effectiveness of biochar on mitigating excessive arsenic (As) accumulation by rice plants was investigated. The treatments were as follows: control, 60 mg kg<sup>-1</sup> As (As applied from NaAsO<sub>2</sub>) and 60 mg kg<sup>-1</sup> As + 20 g kg<sup>-1</sup> biochar. Biochar application to As contaminated soil enhanced the dry weight of rice plants. Dry weight of rice plants decreased by 60 mg kg<sup>-1</sup> As treatment compared to control. Application of As increased As concentration of rice plants while 20 g kg<sup>-1</sup> poultry manure biochar supply to the As contaminated soil significantly decreased the As concentration. Arsenic toxicity induced H<sub>2</sub>O<sub>2</sub> accumulation however the application of biochar reduced H<sub>2</sub>O<sub>2</sub> concentration of plants. Catalase (CAT) and ascorbate peroxidase (APX) activities significantly increased by biochar. Arsenic significantly increased N, S and Zn while decreased Cl, Fe, Cu and Mn concentration of plants. Biochar treatments significantly increased N, P, K and S concentrations of the plants as compared to control and As treated plants. It can be concluded that poultry manure biochar can be used for the prevention of As accumulation in rice plants.

**Keywords:** Antioxidant enzymes; Arsenic toxicity; Biochar.

## Tavuk Gübresi Biyokömürünün Çeltik Bitkisi Arsenik Alımı ve Arsenik Düzeyleri Üzerine Etkisi ve Oksidatif Stres İle İlişkisi

**Öz:** Bu çalışmada, çeltik bitkisinde arsenik (As) birikiminin azaltılması üzerine biyokömürün etkisi araştırılmıştır. Uygulamalar sırasıyla; kontrol, 60 mg kg<sup>-1</sup> As (As NaAsO<sub>2</sub>'den uygulanmıştır) ve 60 mg kg<sup>-1</sup> As + 20 g kg<sup>-1</sup> biyokömür şeklindedir. Biyokömür uygulamasına bağlı olarak, As ile kirlenmiş toprakta yetiştirilen çeltik bitkisinin kuru ağırlığı artış göstermiştir. 60 mg kg<sup>-1</sup> As uygulaması ile çeltik bitkisinin kuru ağırlığı kontrole göre azalmıştır. As uygulaması ile çeltik bitkisinin As konsantrasyonu artarken, 20 g kg<sup>-1</sup> biyokömür uygulaması ile bitkinin As konsantrasyonunu önemli düzeyde azaltmıştır. Arsenik toksisitesi ile bitkide H<sub>2</sub>O<sub>2</sub> birikimi artmış,

ilave edilen biyokömür uygulamasına bağlı olarak  $H_2O_2$  konsantrasyonu azalmıştır. Katalaz (CAT) ve askorbat peroksidaz (APX) aktiviteleri biyokömür uygulaması ile önemli düzeyde artmıştır. Arsenik uygulamasına bağlı olarak çeltik bitkisinin N, S ve Zn konsantrasyonlarını artarken, Cl, Fe, Cu ve Mn konsantrasyonlarını azalmıştır. Kontrol ve As uygulamalarıyla karşılaştırıldığında, biyokömür uygulamasının bitki N, P, K ve S konsantrasyonlarını önemli düzeyde arttırdığı belirlenmiştir. Çeltik bitkisinde As birikimini/toksitesini önlemek için tavuk gübresinden elde edilen biyokömürün kullanılabilceği sonucuna varılmıştır.

**Anahtar Kelimeler:** Antioksidan enzimler, Arsenik toksitesisi; Biyokömür.

## Introduction

Studies on As toxicity have focused mainly on arsenate (AsV) because it is the dominant form in aerobic soils. Anaerobic conditions in paddy soil leads to arsenite (AsIII) mobilization and thus enhanced bioavailability to rice plants (Takahashi et al., 2004; Xu et al., 2008; Su et al., 2010). The reduction of AsV to AsIII is important since AsIII is more toxic to plants and also more water-soluble than AsV (Woolson, 1983). Thermodynamically, reduction of AsV to AsIII can occur quite readily in flooded paddy soils in which bioavailability of As as AsIII increase for rice plants (Zhao et al., 2009). Exposure to As causes plants considerable oxidative stress (Gunes et al., 2009). Arsenite reacts with sulfhydryl groups (-SH) of enzymes and tissue proteins, inhibiting cellular function and causing death (Ullrich-Eberius et al., 1989). Even though As is not a redox metal, there is significant evidence that exposure of plants to inorganic As results in the generation of reactive oxygen species (ROS), which are connected with the valence changes that the element readily undergoes from AsV to AsIII in plants (Meharg and Hartley-Whitaker, 2002). The ROS are strong oxidizing agents that cause oxidative damage (Gunes et al., 2007). ROS produced in plants need to be scavenged for maintenance of normal growth. Plants have evolved mechanisms to protect cells and subcellular systems from the hazardous effects of ROS by using enzymatic antioxidants such as superoxide dismutase, catalase and ascorbate peroxidase (Sairam et al., 2005; Gunes et al., 2007). In our previous work (Gunes et al., 2009), we reported that applied As induced oxidative stress in chickpea plants, and the concentrations of  $H_2O_2$  and lipid peroxidation were increased. However, no information is available considering the effects of biochar treatments on antioxidant enzymes in plants grown in As contaminated soil.

Biochar obtained from poultry manure has potential as a soil amendment, fertilizer and carbon sequestration agent (Lehmann et al., 2011; Gunes et al., 2014). In addition, a number of studies indicate that biochar reduces the phytotoxicity of heavy metals such as Cd, Zn and Pb in the soil. The surface functional groups and adsorption sites on biochar could increase the soil cation exchange capacity and consequently increase metal exchange capacity of soil through the formation of complexes with heavy metals (Beesley et al., 2010, 2011; Cao et al., 2011; Park et al., 2011, Yu et al., 2015). Therefore, application of biochar is a good strategy for remediation of the soils contaminated by heavy metals and other pollutants which renders a reduced risk of toxic metal uptake by plants. Arsenite uptake is of particular importance for rice and other aquatic plants with their roots growing in anaerobic or semi-anaerobic environments. For the reduction of As levels in aerobic conditions some success has been achieved by using biochar in tomato (Beesley et al.,

2013). Unlike AsV, AsIII uptake was not inhibited by phosphate since AsIII may be taken up by aquaporin channels in plant roots (Meharg and Jardine, 2003). Arsenic is known to alter and disturb uptake and transport of nutrients in plants (Päivöke and Simola, 2001). Disturbance of plant mineral nutrition is the main cause for yield decrease, the most frequent sign of As toxicity. There is little information available about As and biochar interactions in flooded conditions (Ma et al., 2014). The objectives of this study were to (1) examine the effects of biochar treatments on As induced oxidative stress, (2) determine the effects of biochar treatments on As and also some essential and non-essential element concentrations of rice plants.

## **Materials and Methods**

### ***Growth Conditions and Treatments***

Rice (*Oryza sativa* L. cv.) plants were grown from June 18, 2014 to July 23, 2014 in a naturally lighted glasshouse (daily average humidity: %57,7; daily average temperature 23.4°C; daily average light: 0.04 MJ/m<sup>2</sup>) at the Faculty of Agriculture, Ankara University, Turkey (39° 57 44.51 N; 32° 51 46.95 E). Some characteristics of the soil used in the experiment and biochar were given in Table 1. Treatments, with four replicates, consisted of control, 60 mg kg<sup>-1</sup> As and 60 mg kg<sup>-1</sup> As + 20 g kg<sup>-1</sup> biochar. Biochar and As levels are based on our previous work (Gunes et al., 2009, 2010). Arsenic was applied as NaAsO<sub>2</sub>. Biochar used in this experiment were derived from pelletized (4.0 mm) poultry manure obtained from caged chicken manure hens by pyrolysis at 300°C under oxygen free conditions for two hours, detailed descriptive information was given in our previous work (Gunes et al., 2014). Mineral element concentrations of poultry manure biochar was determined by polarized energy dispersive XRF (PEDXRF). Biochar and As were applied and incorporated into the soil before seed sowing. For the basal N fertilization, 100 mg kg<sup>-1</sup> of N was applied as NH<sub>4</sub>NO<sub>3</sub> before sowing. Rice seeds were sown at the rate of 20 seeds to each pot which lined with polyethylene and filled with 2 kg of air-dried soil. After a good stand of plants, they were thinned to 13 plants per pot. The pots were flooded with a water layer of 4 cm during the whole growth period. At the end of the experiment, plants were harvested. Shoots were washed once with tap water and twice in deionized water. They were then dried in a forced-air oven at 60 °C until constant mass was reached and they were ground (40 mesh sieve) for elemental analysis.

### ***Enzyme Extraction and Assay***

Samples (500 mg) were taken from the youngest fully emerged leaves and homogenized by a Heidolph, DiAx 900 homogenizer in 5 ml of 100 mM potassium phosphate buffer (pH 7.6) containing 1 mM EDTA-Na<sub>2</sub>. Because APX is labile in the absence of ascorbate, 0.5 mM ascorbate was included for the extraction of this enzyme with the above procedure. The homogenized samples were centrifuged at 10,000 g for 5 minutes. The supernatant was used as a crude enzyme extract in SOD, CAT and APX enzyme analyses. All colorimetric measurements were made at 20 °C in a Shimadzu UV/VIS 1201 spectrophotometer.

**Table 1.** Some physical and chemical properties of the experiment soil and biochar

Physical and Chemical Properties/Methods/Quantification	Amount	
	Soil	Biochar
Field capacity, g kg <sup>-1</sup>	240	-
Texture, Bouyoucos	Clay loam	-
CaCO <sub>3</sub> , g kg <sup>-1</sup>	70.9	-
pH (1:2.5 water)	7.53	10.1
EC (1:2.5 water), dS m <sup>-1</sup>	0.46	12.2
Organic Matter, Walkley Black/g kg <sup>-1</sup>	17.7	-
<b>Nutrition Properties/Methods/Quantification</b>		
Total-N (Kjeldahl), g kg <sup>-1</sup>	0.60	39.0
K (NH <sub>4</sub> OAc-extractable), mg kg <sup>-1</sup>	1068	-
Total-K (X-RF), g kg <sup>-1</sup>	14.7	51.8
Ca (NH <sub>4</sub> OAc- extractable), mg kg <sup>-1</sup>	5559	-
Total-Ca (X-RF), g kg <sup>-1</sup>	36.4	103
Mg (NH <sub>4</sub> OAc- extractable), mg kg <sup>-1</sup>	496	-
Total-Mg (X-RF), g kg <sup>-1</sup>	11.3	7.96
P (NaHCO <sub>3</sub> ), mg kg <sup>-1</sup>	4.98	-
Total-P (X-RF), g kg <sup>-1</sup>	0.91	18.2
Total-S (X-RF), g kg <sup>-1</sup>	0.27	59.6
Zn (DTPA- extractable), mg kg <sup>-1</sup>	0.96	-
Total-Zn (X-RF), mg kg <sup>-1</sup>	70.0	974
Fe (DTPA- extractable), mg kg <sup>-1</sup>	3.62	-
Total-Fe (X-RF), g kg <sup>-1</sup>	39.1	4.83
Cu (DTPA- extractable), mg kg <sup>-1</sup>	1.89	-
Total-Cu (X-RF), mg kg <sup>-1</sup>	36.4	162
Mn (DTPA- extractable), mg kg <sup>-1</sup>	7.55	-
Total-Mn (X-RF), mg kg <sup>-1</sup>	810	1066
Total-Na (X-RF), g kg <sup>-1</sup>	0.37	0.67
Total-Mo (X-RF), mg kg <sup>-1</sup>	4.90	12.5
Total-As (X-RF), mg kg <sup>-1</sup>	12.3	2.40

Superoxide dismutase (SOD) activity was assayed by the nitroblue tetrazolium (NBT) method (Giannopolitis and Ries, 1977). The reaction mixture (3 ml) contained 50 mM Naphosphate buffer, pH 7.3, 13 mM methionine, 75  $\mu$ M NBT, 0.1 mM EDTA, 4  $\mu$ M riboflavin and enzyme extract (0.2 ml). The reaction was started by the addition of riboflavin, and the glass test tubes were shaken and placed under fluorescent lamps (60  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>). The reaction was allowed to proceed for 5 minutes and was then stopped by switching off the light. The absorbance was measured at 560 nm. Blanks and controls were run in the same manner but without illumination and enzyme, respectively. One unit of SOD was defined as the amount of enzyme that produced 50% inhibition of NBT reduction under assay conditions.

Ascorbate peroxidase (APX) activity was determined by following the decrease of ascorbate and measuring the change in absorbance at 290 nm for 1 minute in 2 ml of a reaction mixture containing 50 mM potassium phosphate buffer (pH 7.0), 1 mM EDTA-Na<sub>2</sub>, 0.5 mM ascorbic acid, 0.1 mM H<sub>2</sub>O<sub>2</sub> and 50  $\mu$ L of crude enzyme extract at 25 °C

(Nakano and Asada, 1981). The activity was calculated from the extinction coefficient ( $2.8 \text{ mM}^{-1} \text{ cm}^{-1}$ ) for ascorbate.

Catalase (CAT) activity was determined as a decrease in absorbance at 240 nm for 1 minute following the decomposition of  $\text{H}_2\text{O}_2$ , as modified by Cakmak et al. (1993). The reaction mixture (3 ml) contained 50 mM phosphate buffer (pH 7.0), 15 mM  $\text{H}_2\text{O}_2$  and 50  $\mu\text{L}$  of crude enzyme extract at 25 °C. The activity was calculated from the extinction coefficient ( $40 \text{ mM}^{-1} \text{ cm}^{-1}$ ) for  $\text{H}_2\text{O}_2$ .

### ***Determination of $\text{H}_2\text{O}_2$ Concentration***

The  $\text{H}_2\text{O}_2$  content of fully matured leaves was calorimetrically measured as described by Mukherjee and Choudhuri (1983). To determine  $\text{H}_2\text{O}_2$  levels, leaf samples were extracted with cold acetone. An aliquot (3 ml) of the extracted solution was mixed with 1 ml of 0.1% titanium dioxide in 20% (v:v)  $\text{H}_2\text{SO}_4$  and the mixture was then centrifuged at 6,000 g for 15 minutes. The intensity of yellow color of the supernatant was measured at 415 nm. The concentration of  $\text{H}_2\text{O}_2$  was calculated from a standard curve plotted within the range of 100 to 1000 nM  $\text{H}_2\text{O}_2$ .

### ***Elemental Analysis***

After determination of dry weights, all samples were ground into fine powder in an agate mortar. They were then passed through a 200  $\mu\text{m}$  sieve. Sieved sub-samples were pressed into thick pellets of 32 mm diameter using wax as a binder. USGS Standards, Leaf Standards, GBW 7109 and GBW-7309 sediment as reference standard materials were also pressed into pellets in a similar manner as the samples, and used for quality assurance. Mineral element concentrations were determined by polarized energy dispersive XRF (PEDXRF). The spectrometer used in this study was a Spectro XLAB 2000 PEDXRF spectrometer which was equipped with a Rh anode X-ray tube, 0.5 mm Be side window. The detector of the spectrometer is Si (Li) cooled by liquid  $\text{N}_2$  with a resolution of  $< 150\text{eV}$  at Mn  $K\alpha$ , 5000 cps. The spectrometer configures d as source beam, scattered beam and fluorescent beam all at mutually orthogonal angles (Stephens and Calder, 2004). The sample measurements by PEDXRF were mainly done by three types of targets. The first target (Barkla target) is suitable for the light elements with  $Z > 22$ . The second target (Bragg target) is an oriented crystal target suitable for light elements up to  $Z = 22$ . The third target is a pure metal target suitable for specific elements or small groups of adjacent elements. Additionally this target is useful for generating Compton scatter peaks which can be used for matrix correction (Ivanova et al., 1999; Chuparina and Gunicheva, 2003).

### ***Statistical Analysis***

The experiments were set up in a completely randomized design with five replicate pots. Analysis of variance was performed on the data, and significant differences among treatment means were compared by descriptive statistics ( $\pm$  SE) (MSTAT) and by Least Significant Difference (LSD) and Duncan's multiple range tests (Minitab).

## Results

Dry weight of rice plants was significantly reduced by As treatment (Table 2). However, application of biochar to As contaminated soil significantly recovered the dry weight losses of rice plants. Application of As significantly increased As concentration of rice plants from 2.74 mg kg<sup>-1</sup> to 16.96 mg kg<sup>-1</sup> however, 20 g kg<sup>-1</sup> biochar supply to the As contaminated soil significantly decreased the As concentration to 9.32 mg kg<sup>-1</sup> (Table 3).

**Table 2.** Effect of soil applied biochar on dry weight, H<sub>2</sub>O<sub>2</sub> concentration, and superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) enzyme of rice grown in As contaminated soil. The values are means of 4 replicates. Different letters in each row represent significant difference at the  $P < 0.05$  level based on Duncan's multiple range test.

Parameters	Treatments			<i>F</i> values	<i>LSD</i>
	0 As mg kg <sup>-1</sup>	60 As mg kg <sup>-1</sup>	60 mg kg <sup>-1</sup> As+ 20 g kg <sup>-1</sup> Biochar		
Dry weight (g plant <sup>-1</sup> )	0.46±0.01 a	0.23±0.01 b	0.43±0.05 a	16.4**	0.10
H <sub>2</sub> O <sub>2</sub> (mmol g <sup>-1</sup> fw)	15.8±0.81 b	22.0±1.49 a	14.7±1.27 b	10.4**	3.77
SOD (Unit mg <sup>-1</sup> fw)	88.0±7.95 a	61.2±0.98 b	67.3±1.83 b	8.77**	14.62
CAT (mmolg <sup>-1</sup> min <sup>-1</sup> fw)	0.18±0.01 b	0.19±0.01 b	0.32±0.02 a	42.6**	0.04
APX (mmolg <sup>-1</sup> min <sup>-1</sup> fw)	3.38±0.57 b	2.98±0.38 b	5.11±0.29 a	6.95**	1.33

Significance of ANOVA: \*\*:  $P < 0.01$ .

**Table 3.** Effect of soil applied biochar on As, N, P, K, Ca, Mg, S, Fe, Zn, Cu, Mn and Mo concentration of rice plants grown in As contaminated soil. The values are means of 4 replicates. Different letters in each row represent significant difference at the  $P < 0.05$  level based on Duncan's multiple range test.

Parameters	Treatments			<i>F</i> values	<i>LSD</i>
	0 As mg kg <sup>-1</sup>	60 As mg kg <sup>-1</sup>	60 mg kg <sup>-1</sup> As+ 20 g kg <sup>-1</sup> Biochar		
As (mg kg <sup>-1</sup> )	2.74±0.36 c	17.0±1.37 a	9.32±0.91 b	53.9**	2.98
N (g kg <sup>-1</sup> )	23.1±0.16 c	28.5±1.39 b	52.0±2.13 a	108.9**	4.52
P (g kg <sup>-1</sup> )	2.52±0.06 ab	2.30±0.06 b	2.71±0.09 a	7.84**	0.22
K (g kg <sup>-1</sup> )	43.6±0.15 b	42.0±0.60 b	46.2±0.63 a	14.1**	1.74
Ca (g kg <sup>-1</sup> )	5.58±0.20	6.01±0.43	5.10±0.39	1.64 <sup>ns</sup>	-
Mg (g kg <sup>-1</sup> )	2.52±0.16	2.29±0.20	2.51±0.09	0.69 <sup>ns</sup>	-
S (g kg <sup>-1</sup> )	2.82±0.07 c	4.31±0.21 b	5.59±0.30 a	41.2**	0.66
Cl (g kg <sup>-1</sup> )	15.6±0.36 a	14.3±0.40 b	15.6±0.21 a	5.51**	1.03
Fe (mg kg <sup>-1</sup> )	614±56.9 a	309±11.0 b	552±12.7 a	22.0**	105
Zn (mg kg <sup>-1</sup> )	29.7±1.37 b	80.0±13.10a	66.6±14.7a	5.20**	35.2
Cu (mg kg <sup>-1</sup> )	19.1±2.62 a	12.3±0.78 b	12.4±0.91 b	5.44**	5.11
Mn (mg kg <sup>-1</sup> )	480±11.5 a	351±12.9 b	481±17.2 a	28.2**	43.4
Mo (mg kg <sup>-1</sup> )	3.58±0.65	5.72±0.53	4.44±0.75	2.77 <sup>ns</sup>	-

Significance of ANOVA: \*\*:  $P < 0.01$ ; ns: non-significant

Arsenic treatment increased  $H_2O_2$  concentrations while the application of biochar significantly reduced  $H_2O_2$  concentration of rice plants. The SOD activity was decreased by As toxicity and application of biochar had no effect on SOD activity. However, CAT and APX activities significantly increased by biochar treatments (Table 2).

Arsenic toxicity significantly increased N, S and Zn concentrations while decreased Cl, Fe, Cu and Mn concentrations of rice plants (Table 3). The concentrations of P, K, Ca, Mg and Mo were not changed by As treatment. Biochar treatments significantly increased N, P, K and S concentrations of the plants as compared to control and As treated plants. Both As and As+Biochar treatments had no significant effect on Ca, Mg and Mo concentrations of rice plants. Arsenic induced reductions in Cl, Fe and Mn concentrations was increased again by biochar treatments.

## Discussion

Application of  $60 \text{ mg kg}^{-1}$  As to soil caused phytotoxicity for rice plants. Phytotoxicity of As has also been previously reported for fern (Tu and Ma, 2003; Tu et al., 2004), chickpea (Gunes et al., 2009) and sunflower (Gunes et al., 2010). Biochar treatments reduced As toxicity by reducing As concentrations of rice plants. The reduction of As in plant tissues is the reason of reduced As availability in soil by applied biochar. Beesley and Marmiroli (2011) reported that applied biochar to As contaminated soil reduces mobility of As in soil. The mechanism here, according to Beesley et al. (2014), is that biochars due mainly to their ability to sorb metals reduces their phytotoxicity. For the pollution control, heavy metal and As removal from waste-waters (Mohan et al., 2007) and soil leachates (Fellet et al., 2011) by biochar amendments have been reported. In addition to this, Beesley et al. (2011) suggested Fe oxide and other As immobilizing material stating this may also mean that those material conditions induced by biochar addition to soils, which may not necessarily impact on metal mobility, could control As mobility, regardless of the capacity of biochar as a sorbent. Gregory et al. (2015) suggested that increased microbial activity after biochar treatment reduces As solubility in the soil. The reason underlying in As concentration reduction in rice plant tissue so, in turn, As toxicity reduction by biochar application in the present study might probably be the end-results of As mobility reduction by biochar and/or As sorption to biochar. Application of As increased the accumulation of  $H_2O_2$  in plant tissues while applied biochar reduced the level of  $H_2O_2$  by increased CAT and APX activities. Responses of plants to toxic metals are complex and several defense strategies have been suggested such as complexation of ions, reduced influx, and enhanced production of antioxidants that detoxify ROS that are produced in response to the metals (Meharg, 1994). This is a process that readily occurs in plants (Stoeva et al., 2003). Although As is not a redox metal, there is significant evidence that exposure of plants to As results in the generation of ROS, which is connected with As valence change (Gunes et al., 2009). As plants have antioxidant enzymes to combat oxidative stress, altered activities of these systems are also frequently used as indicators of oxidative stress in plants (Mittler, 2002). SOD catalyzes the conversion of highly reactive superoxide ( $O_2^{\bullet-}$ ) to  $H_2O_2$  and, hydrogen peroxide is scavenged by CAT and APX. The CAT and APX activities of the rice plants were significantly increased by biochar treatment, since these plants contained increased concentrations of  $H_2O_2$ . Both As and As+Biochar treatments decreased SOD activities, similar results for different types of oxidative stress and As toxicity have also

been reported previously (Mishra and Choudhuri, 1999; Gong et al., 2005; Gunes et al., 2009).

Arsenic-induced ionic imbalances have not been widely studied. However, Fayiga et al. (2008) published a work describing effects of Ca, K, P, NH<sub>4</sub>, and Cl on As accumulation in *Pteris vittata* L. and (Hartley and Lepp, 2008) studied the effect of Fe oxide on the As uptake of spinach and tomato. In these works, Ca, K, P, NH<sub>4</sub>, Cl, and Fe oxides were found to reduce As uptake of plants. In the present study, application of As gave increased concentrations of N, S, Zn and Mo in rice plants. These increases can be attributed to the concentration effect in the plants because of growth reduction caused by As toxicity. In contrast to this, As reduced Cl, Fe, Cu and Mn concentration of rice plants. Interaction between As and Fe previously reported by Hartley and Lepp (2008) and Das et al. (2008). They suggested that the As contamination in soils may be reduced by applying Fe oxides to the soil. However, it is more difficult to postulate how the presence of As could influence uptake of the other elements in soil. The most important finding of this work is that the biochar application to As contaminated soil reduced As concentrations while increased N, P, K, S, Cl, Fe and Mn concentrations of rice plants. The effect of biochar on As contaminated soil with rice crop was first reported in this study. In our previous study which was carried out non As contaminated soil, application of biochar improved lettuce growth and N, P and K nutrition, but it reduced Fe, Cu, Zn and Mn nutrition of lettuce (Gunes et al., 2014). In our latest work, again applied biochar applications increased the growth of maize and bean plants and biochar applications resulted in increased concentrations of N, P, K, Zn, Cu and Mn in maize and bean plants (Inal et al., 2015).

## Conclusion

To our knowledge, the above observations are the first report on the influence of biochar application on As accumulations in rice plant. Applied biochar significantly reduced As concentration of rice plants, probably through making As in the soil low available. Arsenic application reduced plant growth and caused mineral imbalances however biochar application improved antioxidant mechanisms and mineral nutrition of As treated rice plants. It was hoped that this study provides a basis for developing strategies for the reduction of the risk associated with As toxicity and these promising results now need to be complemented by field and long-term studies.

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