RESEARCH ARTICLE

Analysis of Arsenic Accumulation and its Effects on the Ionome Profile of Rice (*Oryza sativa* L.) Plants

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ABSTRACT

The accumulation of arsenic (As), a toxic carcinogenic element, in rice plants is a matter of significant environmental and human health concern. This study was performed to analyze the impact of As on ionome profile of rice (*Oryza sativa* L.) plants. The rice seedlings were subjected either to fixed concentration of 20 µM arsenite [As(III)] for different durations (1, 3, 7, 15 and 30 d) or to different concentrations of As(III) (0, 3, 5, 10, 20, and 50 µM) for fixed duration of 15 d. In both concentration- and duration-dependent experiments, As concentration in leaves and roots was found to increase progressively. The maximum As level was observed at 50 µM in concentration dependent experiment (185 µg g^{-1} dw in leaves and 9027 µg g^{-1} dw in roots) and at 30 d in duration dependent experiment (78 µg g^{-1} dw in leaves and 6175 µg g^{-1} dw in roots). In concentration dependent experiment, Ni showed a progressive increase while Cu (at all concentrations) and Mn (beyond 5 µM) a decline in both leaves and roots. Zn and Co showed an increase in leaves while a decline in roots. A similar trend of different element concentration was recorded in duration dependent experiment. The present analyses thus highlight that As exposure has profound influence on elemental composition of rice seedlings. Therefore, the health and safety aspects of As impacted rice plants must also be assessed from the perspective of other elemental concentrations.

Keywords: Arsenic, Copper, Ionome, Manganese, Rice, Zinc.

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INTRODUCTION

The arsenic (As) contamination of the soil and water in several states of India including West Bengal, Bihar and Uttar Pradesh is a serious problem (Upadhyay *et al*., 2019a). It not only affects the water and soil quality but also threatens human health via drinking water and food (Upadhyay *et al*., 2019b). Rice is a widely cultivated crop plant in the As affected regions of India and is also hugely consumed by people. Rice is known to accumulate As in significant amounts in its tissues and grains (Awasthi *et al*., 2017). This is primarily because of presence of selective and highly expressed silicic acid transporters in rice plants (Srivastava *et al*., 2016). Arsenic takes entry into rice plants via these transporters in the form of arsenite [As(III))]. Further, the cultivation of rice in submerged conditions leads to conversion of most of the As in the form of As(III). The other important contributing factors influencing As accumulation in rice include iron plaque formation and radial oxygen loss (Wu *et al*., 2011; Awasthi *et al*., 2017).

The growth of rice plants is affected by As accumulation and a number of physiological, biochemical and molecular changes have been observed (Srivastava *et al*., 2016; Chauhan *et al*., 2017). The exact mechanism of growth effects is not yet fully elucidated. It is well known that several essential elements like zinc (Zn), copper (Cu), manganese (Mn), iron (Fe) etc. play vital role in plant growth and developmental processes through involvement in a number of biochemical reactions. Therefore, one likely explanation for the As-induced growth retardation of rice plants has been the impact of As on mineral element levels (Chauhan *et al*., 2017). Earlier, Dwivedi *et al*. (2010) have performed simulated pot experiments with four rice genotypes and found that As exposure caused reduction in elemental concentrations (Fe, Zn, Mn, Cu) at high As dose. The ionome refers to the mineral nutrient and trace element composition of a plant and the ionomics experiments pertain to the quantitative measurement of a whole set of elementsduring the growth cycle of a plant or in response to an abiotic or biotic stress (Salt *et al*., 2008). However, the targeted studies on ionome profiling of rice plants in response to As stress are limited and more research on this aspect is needed. This study was therefore planned to assess the ionome profile of rice plants in response to As stress imposed in concentration dependent and duration dependent manner.

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MATERIALS AND METHODS

Rice (*Oryza sativa* L.) seeds of variety IR64 were grown in ½ strength kimura nutrient medium in glass beakers in a Plant Growth Chamber (Sanyo, Japan) as per the procedure detailed previously (Srivastava *et al*., 2016). Seeds were surface sterilized with 30% ethanol for 3 min and then washed thoroughly with distilled water to remove traces of ethanol. After incubation period (14-16 h), seeds were germinated on a Petri plate under dark conditions. The seedlings were grown for 12 d in control conditions in beakersin ½ Kimura solution (pH 5.5) with a daily cycle of a 14 h photoperiod with a light intensity of 150 µE m^{-2} s^{-1}, day/night temperature of 25/22°C and relative humidity of 65-75%. At 12th d, rice seedlings were subjected either to fixed concentration of 20 µM As(III) ($NaAsO_2$) for different durations 1, 3, 7, 15 and 30 d or to varying concentrations of As(III) (0, 3, 5, 10, 20 and 50 µM) for fixed duration of 15 d. At the harvesting time point, seedlings were separated into roots and leaves, which were kept in a hot air oven for drying at 80ºC. The level of various elements (As, Mn, Ni, Co, Cu and Zn) was analysed by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). For each treatment, seedlings were washed thoroughly in ice-cold milli-Q water to remove adsorbed As. The dried tissue (~100 mg) was kept in 1 mL of concentrated HNO_3 overnight at room temperature and then digested at 120°C. The residue was then diluted in 10 mL of milli-Q water and subjected for elemental estimation using ICP-MS (Thermo Scientific Q-ICP-MS, X Series 2). The standard reference materials of metals were used for calibration and quality assurance for each analytical batch. For accuracy, repeated analysis (n=6) of quality control samples was done. All the treatments were performed in five replicates.

RESULTS

Concentration-dependent experiment

ICP-MS analysis showed that As accumulation by rice plants was a concentration dependent phenomenon. In concentration dependent experiment, As level in both leaves and roots of rice seedlings increased with the increase in As(III) concentration. The maximum As accumulation in rice leaves was found to be 185 µg g^{-1} dw while it was 9027 µg g^{-1} dw in roots (Fig. 1A & 2A). The level of Mn in leaves and roots showed an increase up to 5 µM (263 µg g^{-1} dw; 17% increase in leaves and 446 µg g^{-1} dw; 47% increase in roots, in comparison to control) followed by a decline up to 50 µM (50 µg g^{-1} dw; 78% decline in leaves and 101 µg g^{-1} dw; 67% decline in roots, in comparison to control) (Fig. 1B & 2B). The level of Ni showed an increase with the increase in As(III) exposure concentration in both leaves (26.95 µg g^{-1} dw at 50 µM; 180% increase in comparison to control) and roots (406 µg g^{-1} dw at 50 µM; 120% increase in comparison to control) (Fig. 1C & 2C). The level of Co increased in all As(III) concentrations in comparison to control in leaves with the maximum increase occurring at 20 µM at 15 d (0.64 µg g^{-1} dw; 123% increase). However, in roots, Co level declined beyond 5 µM with the maximum decline at 50 µM (4.06 µg g^{-1} dw; 56% decrease in comparison to control) (Fig. 1D & 2D).

The level of Cu showed a progressive decrease in response to As(III) stress in both leaves (12.86 µg g^{-1} dw at 50 µM; 59% decrease in comparison to control) and roots (44.74 µg g^{-1} dw at 50 µM; 67% decrease in comparison to control) (Fig. 1E & 2E). The level of Zn showed a significant increase in comparison to control in leaves (1243 µg g^{-1} dw at 50 µM; 319% increase) while a decline in roots (the maximum decline at 20 µM, 233 µg g^{-1} dw; 66% decrease) (Fig. 1F & 2F).

Duration-dependent experiment

In duration dependent experiment also, As accumulation increased with an increase in duration with the maximum being at 30 d in both leaves (78 µg g^{-1} dw) (Fig. 3A) and roots (6175 µg g^{-1} dw) (Fig. 4A). The level of Mn showed a decline in response to As(III) exposure in comparison to control in leaves, however, in roots, Mn level showed a decline beyond 3 d. The maximum decline of 47% and 58% was observed in leaves and roots, respectively at 15 d (Fig. 3B & 4B). The level of Ni increased significantly in response to As(III) on all durations in leaves with the maximum increase being at 30 d (25.63 µg g^{-1} dw; 312%). However, in roots, Ni content did not show a significant increase but a decline occurred at 3, 7 and 30 d. The maximum decline in Ni content occurred at 30 d (41%) in comparison to control (Fig. 3C & 4C). The Co content in leaves was increased non-significantly or significantly on all durations with the maximum increase being at 15 d (88% in comparison to control). In roots, Co content showed significant decline on all durations except at 1 d with the maximum decline being at 15 d (75% in comparison to control) (Fig. 3D & 4D). The Cu level showed a significant decline in comparison to control in both leaves except at 1 and 3 d in leaves. The maximum decline in Cu level occurred at 30 d in both leaves (11.77 µg g^{-1} dw, 61% decline) and roots (67.94 µg g^{-1} dw, 54% decline) (Fig. 3E & 4E). The level of Zn showed contrasting responses in leaves and roots. In leaves, Zn level showed an increase in comparison to control on all duration and the increase became more with the duration, while in roots a reverse trend was observed. The maximum increase in leaves (86%) was observed at 15 d while the maximum decline in roots (76%) occurred at 30 d.

DISCUSSION

The maintenance of optimal concentrations of various essential major and trace elements is of crucial importance for proper growth and development of plants. The process requires concerted action of several transporters for the uptake of element at roots and translocation to different plant parts. The process of elemental nutrition is threatened by varying bioavailability of various essential metals and also due to presence of toxic elements like As at certain places. Arsenic uptake and translocation by plants depends on the species of As present in soil and involves role of transporters like aquaglyceroporins, phosphate transporters, NRAMPs and ATP-Binding Cassette transporters (Awasthi *et al*., 2017; Kumari *et al*., 2018). The uptake of As by these transporters occurs via competition with other essential elements like silica (Si), boron (B) and iron (Fe). In addition, the detoxification of As within the plant requires involvement of essential elements like sulphur (S) and nitrogen (N) (Srivastava *et al*., 2016; 2019). Further, As induced influence on plant growth and root phenotype (Srivastava *et al*., 2019) leads to disturbances in plants' capacity to maintain optimal elemental nutrition (Chauhan *et al*., 2017).

The results of the present study depicted that As accumulation increased in rice seedlings leaves and roots in concentration- and duration-dependent manner. Arsenic accumulation by rice plants is a well-known phenomenon (Dixit *et al*., 2015; Srivastava *et al*., 2016). As a consequence of increased As accumulation, altered levels of analysed elements were observed. The level of Mn and Cu showed a decline in both leaves and roots and in both concentration dependent and duration dependent experiments. Mn and Cu are essential elements required for normal plant growth and development. Thesearean essential component of oxygen-evolving and water-splitting complex of photosystem II (PSII) (Nickelsen and Rengstl, 2013). Mn is also essential part of several steps of carbohydrate, lipid and lignin biosynthesis in plants (Marschner, 2012). Further, Mn is a co-factor of several enzymes (~35) in plants like RNA polymerases, glycosyltransferases and superoxide dismutase (MnSOD) (Hebbern *et al*., 2009; Marschner, 2012; Socha and Guerinot, 2014). The deficiency of Mn can lead to interveinal chlorosis, and decrease in net photosynthesis (Socha and Guerinot, 2014) and such symptoms are a common response to As stress in rice plants (Srivastava *et al*., 2013). Cu is an essential element participating in photosynthetic electron transport, mitochondrial respiration, oxidative stress responses and is a cofactor in enzymes such as Cu/Zn superoxide dismutase (SOD), cytochrome c oxidase, amino oxidase, plastocyanin and polyphenol oxidase (Yruela, 2005). The deficiency of Cu is linked to disturbed redox status, electron transport and increased production of reactive oxygen species (ROS) (Yruela, 2005). Arsenic is known to induce the production of ROS and cause oxidative stress to plants (Mylona *et al*., 1998). Thus,

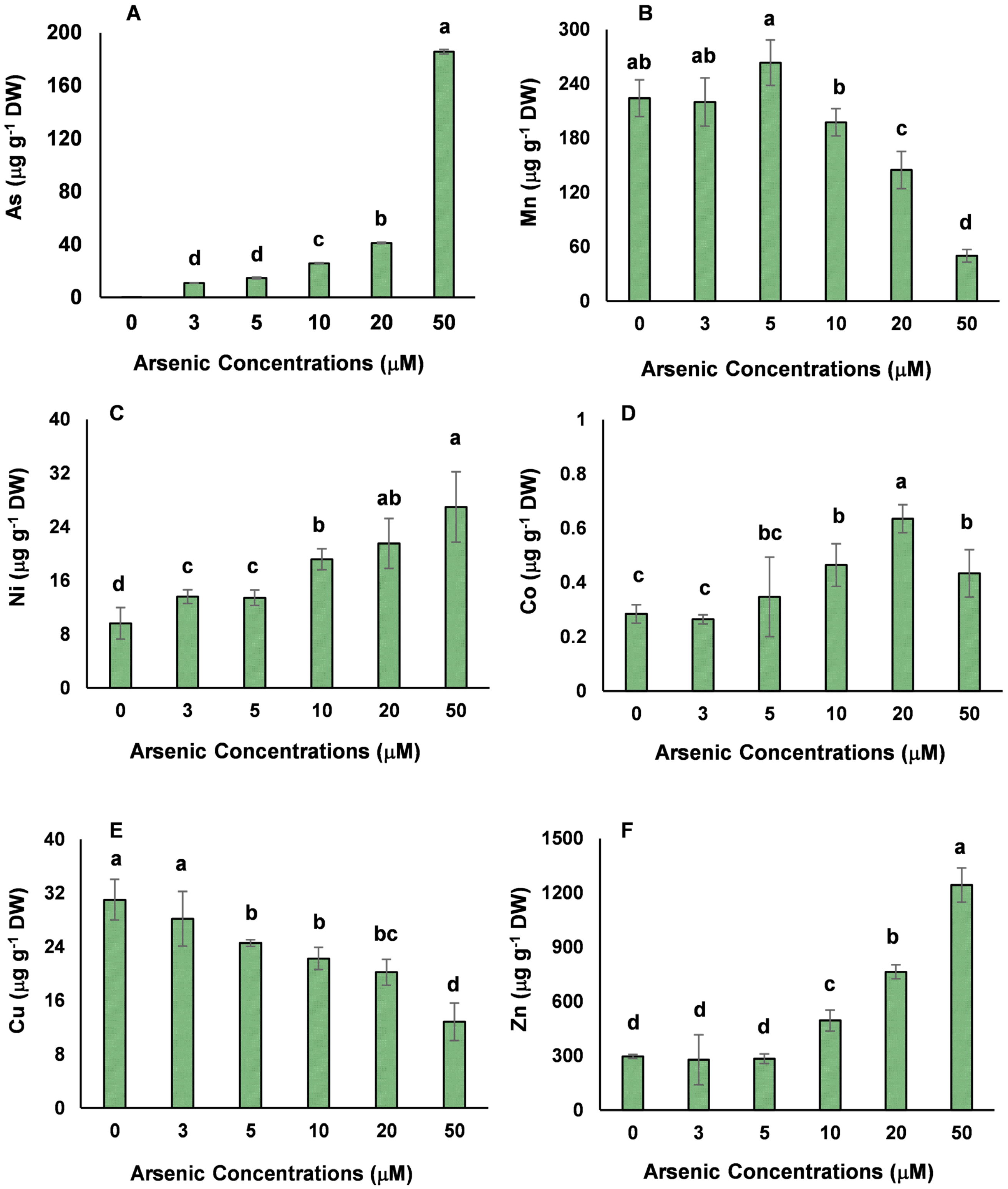

Fig. 1: The effect of different exposure concentration of arsenite on the accumulation (µg g^{-1}dw) of As (A), Mn (B), Ni (C), Co (D), Cu (E) and Zn (F) in leaves of rice seedlings after 15 d. All values are means of triplicates ± SD. ANOVA significant at $p \leq 0.01$. Different letters indicate significant difference between means (DMRT, P <0.05).

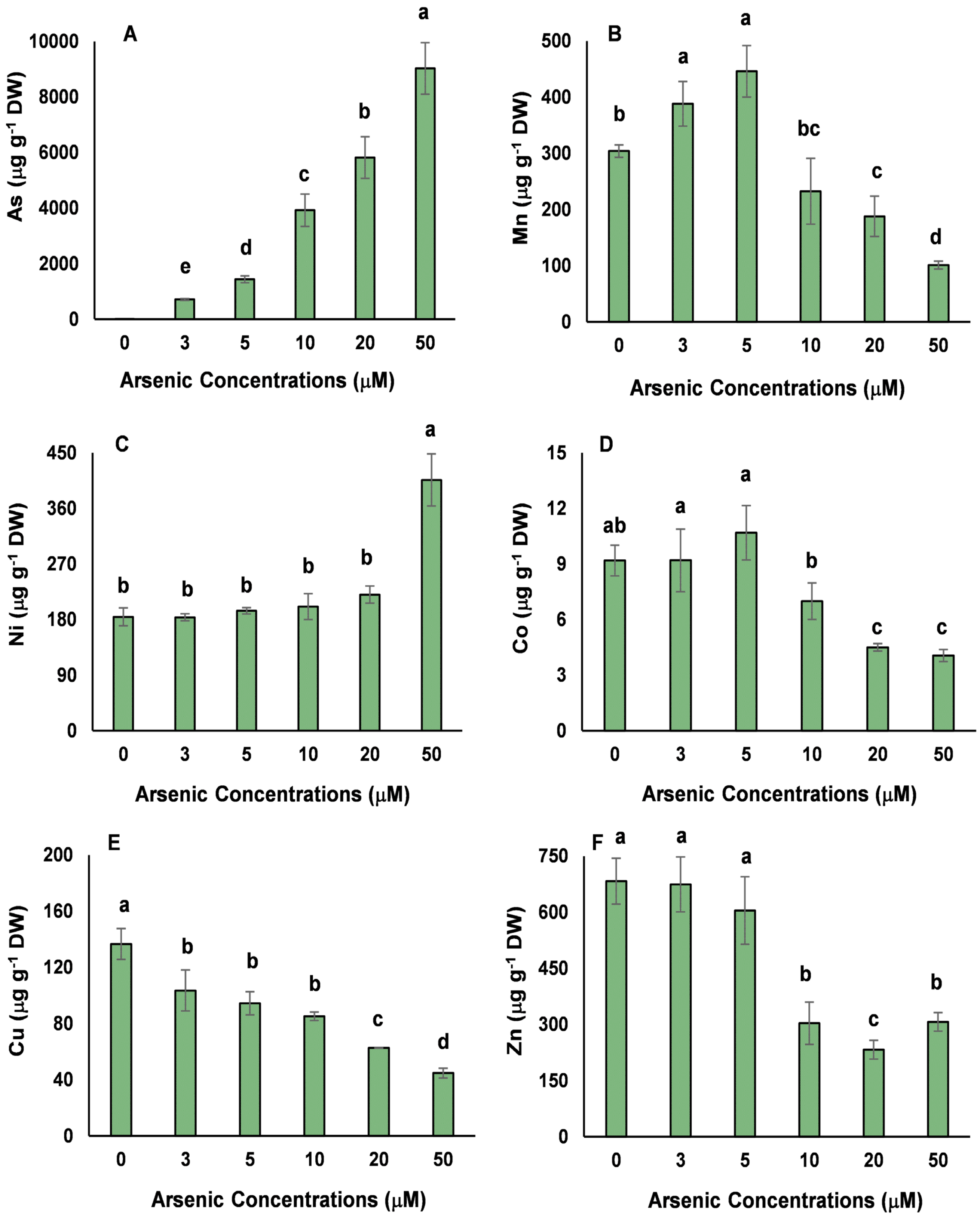

Fig. 2: The effect of different exposure concentration of arsenite on the accumulation (μg g^{-1} dw) of As (A), Mn (B), Ni (C), Co (D), Cu (E) and Zn (F) in roots of rice seedlings after 15 d. All values are means of triplicates ± SD. ANOVA significant at $p \leq 0.01$. Different letters indicate significant difference between means (DMRT, P <0.05).

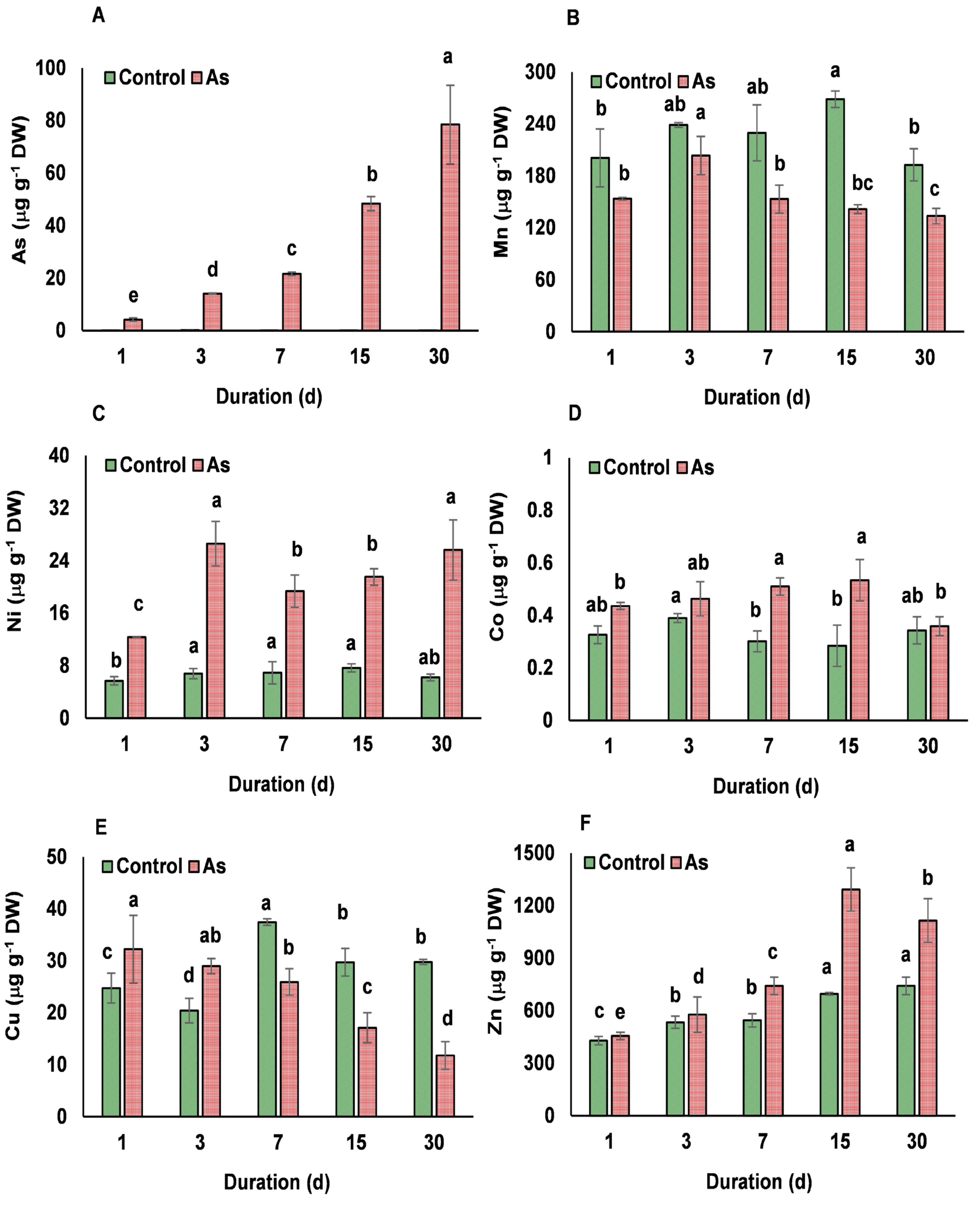

Fig. 3: The effect of 20 μM arsenite on the accumulation (μg g^{-1} dw) of As (A), Mn (B), Ni (C), Co (D), Cu (E) and Zn (F) in leaves of rice seedlings after different times points. All values are means of triplicates ± SD. ANOVA significant at $p \leq 0.01$. Different letters indicate significant difference between means for a particular treatment (DMRT, P <0.05).

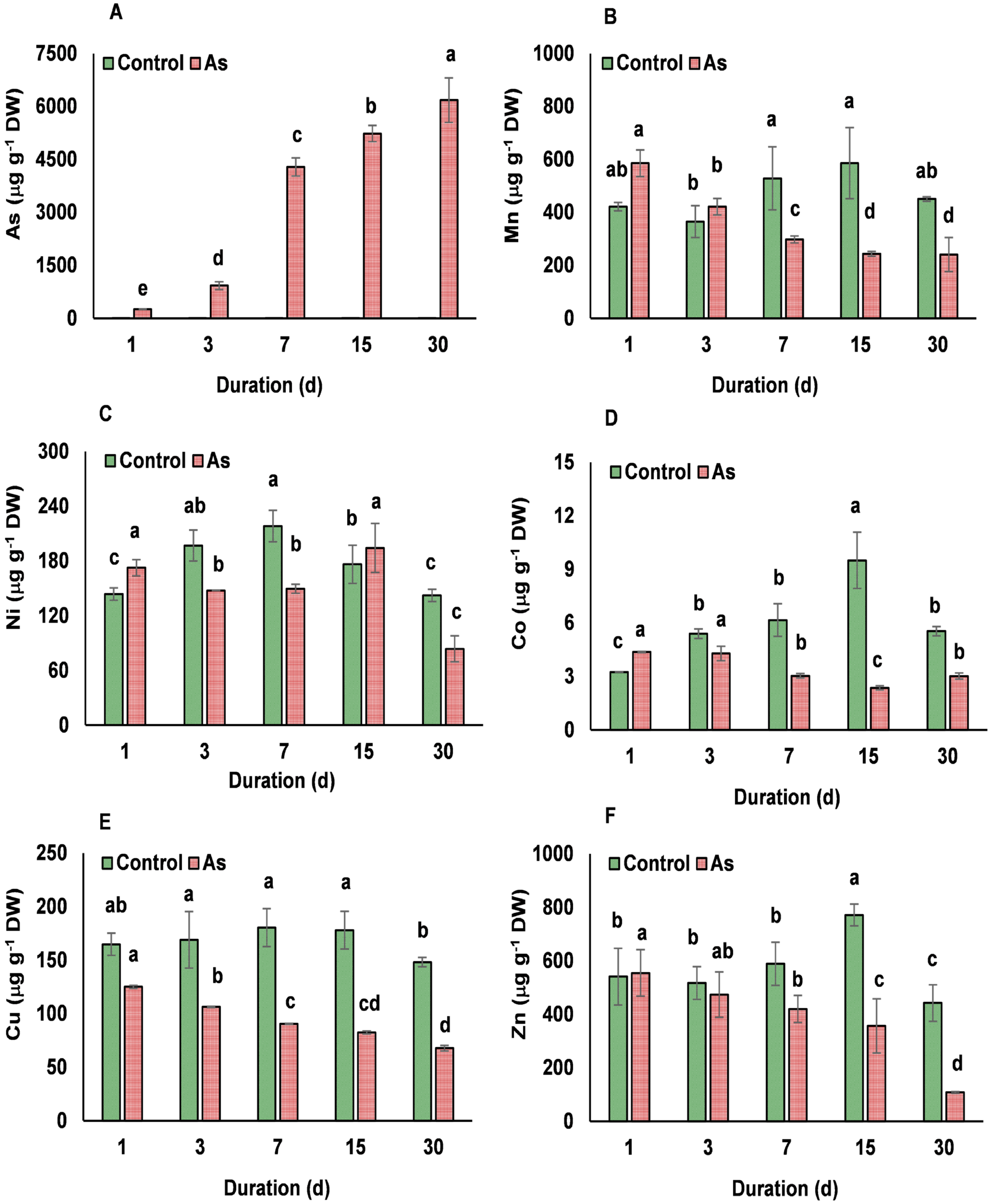

Fig. 4: The effect of 20 µM arsenite on the accumulation (µg g^{-1} dw) of As (A), Mn (B), Ni (C), Co (D), Cu (E) and Zn (F) in roots of rice seedlings after different times points. All values are means of triplicates ± SD. ANOVA significant at $p \leq 0.01$. Different letters indicate significant difference between means for a particular treatment (DMRT, P <0.05).

Mn and Cu deficiency induced by As accumulation might be linked to As stress induced toxicity to plants.

The level of Zn and Co showed contrasting responses with increase in leaves while a decline in roots. It thus appears that plants' attempted to increase Zn and Co concentration in leaves either by increasing the uptake of element via roots or by enhancing element translocation from roots to leaves. Zn is an essential element for normal cell functions (Cakmak, 2000). Zn is involved in carbohydrate metabolism, carbonic anhydrase, antioxidant enzymes (Cu/Zn SOD), stabilization of ribosomal fractions, and cytochrome synthesis (Hafeez *et al*., 2013). Zn is also known to interact with NADPH oxidase so as to prevent oxidative damage to critical cell components. Further, via balancing of Fe nutrition, Zn helps in reduction of ROS production.In an earlier study, Zn supplementation along with As has been found to impart stress tolerance to *Hydrilla verticillata* plants. The effects were mediated through improved antioxidant potential and thiol metabolism (Srivastava and Shrivastava, 2017). In rice also, the application of Zn has been found to reduce As toxicity (Das *et al*. 2005). Thus, increased Zn level in leaves might be to avoid excessive ROS production and protect photosynthetic functions. In the conditions of Cu deficiency, increase in Zn might be an adaptive response of plants that needs to be studied further. Co concentrations in plants range from 0.1 to 10 μg g^{-1} dw, as observed in this study also (Jaleel *et al*. 2009). Although Co was not a component of nutrient medium (Kimura medium) in the present experiment, it may have been present in certain quantity due to minute impurities in various chemicals. The mobility of Co from root to shoot is low (Palit *et al*., 1994). In this work also, the level of Co in roots was about 10-20 fold higher than that in leaves. Co is not an essential element for plants except nitrogen fixation by legumes; however low Co concentrations promote plant growth (Marschner, 1995; Jaleel *et al*., 2009). Nonetheless, excess Co concentrations are reported to be toxic to plants causing loss to leaf pigments (Rancelis *et al*., 2012) and inhibit carbon fixation (Palit *et al*., 1994). Hence, the observed increase in Co uptake by plants in leaves in response to As stress might be linked to toxicity of As promoting Co increase in leaves. This needs to be investigated further in future research.

In the present experiments, Ni level was found to significantly increase in leaves and roots of rice seedlings in response to As stress in concentration dependent experiment but only in leaves in duration dependent experiment. Ni is an essential micronutrient for plants, though only at low levels up to 5 μg g^{-1} dw (Ahmad and Ashraf, 2011; López and Magnitskiy, 2011). It is a constituent of enzymes like urease, Ni-Fe hydrogenase, Ni-SOD, acetylcoenzyme A synthase, and hydrogenases (Marschner, 1995; Negi *et al*., 2014). Further, Ni is also important for the synthesis of anthocyanins (López and Magnitskiy, 2011). However, high Ni level can cause toxicity to plants and can interfere with mineral absorption by roots, photosynthesis, stomatal conductance and transpiration, plant water relations, and enzyme activities (Yusuf *et al*., 2011; Negi *et al*., 2014; Anjum *et al*., 2015). Excess Ni can also induce ROS production and alter antioxidant enzyme activities (Gajewska and Sklodowska, 2007; Gajewska *et al*., 2012; Nasibi *et al*., 2013). In this study, the accumulation of Ni to high levels (up to 27 μg g^{-1} dw in leaves and 406 μg g^{-1} dw in roots) appears to be linked to As toxicity mechanism. As exposure is known to induce anthocyanin production in rice seedlings (Srivastava *et al*., 2016) and this might be linked to increased Ni level observed in this study.

In conclusion, the altered profile of essential mineral elements like Cu, Zn and Mn and beneficial elements like Co and Ni were apparent to be toxicity response of plants to As(III) exposure. However, the changes in level of Co and Ni need to be studied further from the perspective of adaptive response of plants to alter plant metabolism so as to tolerate As stress.

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