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ScienceDirect

Rice Science, 2018, 25(5): 235−249

Arsenic Stress Responses and Tolerance in Rice: Physiological, Cellular and Molecular Approaches

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Abstract: Arsenic (As), a potentially toxic metalloid released in the soil environment as a result of natural as well as anthropogenic processes, is subsequently taken up by crop plants. In rice grains, As has been reported in Asia, North America and Europe, suggesting a future threat to food security and crop production. As^{3+} by dint of its availability, mobility and phytotoxicity, is the most harmful species of As for the rice crop. Specific transporters mediate the transport of different species of As from roots to the aboveground parts of the plant body. Accumulation of As leads to toxic reactions in plants, affecting its growth and productivity. Increase in As uptake leads to oxidative stress and production of antioxidants to counteract this stress. Cultivars tolerant to As stress are efficient in antioxidant metabolism compared to sensitive ones. Iron and selenium are found to have ameliorating effect on the oxidative stress caused by As. Microbes, even many indigenous ones, in the plant rhizosphere are also capable of utilizing As in their metabolism, both independently and in association. Some of these microbes impart tolerance to As-stress in plants grown in As contaminated sites.

Key words: arsenic; rice; phytotoxicity; hyperaccumulator; phytochelatin; antioxidant; mitigation

Arsenic (As), a potentially toxic metalloid, is a naturally occurring element ubiquitous to all soils (Smedley and Kinniburgh, 2002; William et al, 2005). It is the 20th most common element in the earth's crust (Mandal and Suzuki, 2002). Soil contains 1.5–3.0 mg/kg As. As is present in inorganic forms in various minerals in soil, the important ones being realgar, arsenopyrite, etc. From the minerals, As in both inorganic and organic forms, gets mobilized due to natural and human activities, and becomes more readily available to living organisms.

Among the natural sources of mobilization of As, weathering of As-containing minerals (Woolson, 1977; Smedley, 2006) and the activities of microorganisms (Bentley and Chasteen, 2002; Turpeinen et al, 2002) are prominent. On methylation by microbes, As is released as monomethyl arsenate (MMA) or dimethyl arsenate (DMA) (Bentley and Chasteen, 2002). Some microbes utilize As in their metabolism, and release the toxic trimethyl arsine oxide (TMAsO) gas which is subsequently released to the atmosphere (Mandal and Suzuki, 2002).

Human activities that release more As in bio-available forms include application of As pesticides, use of As in paints to be subsequently released by molds and bacteria, mining of metals and heavy extraction of groundwater. Insecticides [calcium arsenate ($Ca_3As_2O_8$) and lead arsenate ($PbHAsO_4$)], herbicide [sodium arsenite ($NaAsO_2$)], rodenticides arsenious oxide (As_2O_3) and sodium arsenite ($NaAsO_2$) contain As (ICAR, 2009). Their residues remain in the soil, get dissolved in the groundwater and become easily available to living organisms. As-containing wallpaper paints, fed upon by molds and bacteria, led to release of the 'Gosio' gas, which was identified to be trimethyl arsine (Woolson, 1977). Metal mining,

Received: 28 February 2018; **Accepted:** 19 June 2018

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Peer review under responsibility of China National Rice Research Institute

http://dx.doi.org/

processing of ores and related activities, has contributed to the release of As in the soil and groundwater (Smedley, 2006) in several countries including Thailand, Ghana, Turkey, England, Serbia, Bosnia, Poland, USA and Canada (Barringer and Reilly, 2013).

A very significant means of mobilization of As is the extraction of groundwater from shallow aquifers in various countries, where surface water is contaminated by disease causing microorganisms. Groundwater is generally more vulnerable to As contamination than surface water because of the interaction of groundwater with aquifer minerals and the increased potential in aquifers for the generation of the physicochemical conditions favourable for As release (Smedley, 2006). Groundwater obtained from shallow aquifers is more likely to contain higher amount of As than that obtained from deep aquifers (Smedley, 2006). As contaminated groundwater is found in many countries like Bangladesh, India, China, Argentina, Chile, Vietnam, Hungary and Mexico (Smedley and Kinniburgh, 2002; Smedley, 2006; Chakraborti et al, 2012). Deep aquifers containing high As levels have also been reported in the Mekong Valley, Vietnam, where high amount of groundwater extraction has resulted in subsidence of the land level by almost 3 cm per year as measured by satellite based radar images from 2007 to 2010 (Erban et al, 2013), leading to speculations that similar deep aquifers of groundwater may not remain As free over the years to come.

In soil, As exhibits a number of oxidation states, the common ones being As^{5+}, As^{3+} and As^{3-}. In aerobic soils, As is mainly present in the oxidized form as arsenate (As^{5+}). While in anaerobic environments like paddy soil, it mainly exists in the reduced form as arsenite (As^{3+}) (Takahashi et al, 2004).

With the use of As-contaminated water in irrigation and due to the various processes of its mobilization, the levels of As in soils have escalated, affecting the agricultural system and resulting in the uptake of the element by crop plants. The translocation factor (TF) for As is higher in rice (0.8) compared to the other crops like wheat (0.1) and barley (0.2) (Xu et al, 2008). The amount of As in paddy soil and soil solution stand elevated (Brammer and Ravenscroft, 2009). The scenario is the worst in rice fields in affected areas such as Bangladesh, India, China and Thailand, where rice is the staple food for most of the people (Abedin et al, 2002; Williams et al, 2006; Garnier et al, 2010). Humans also have been exposed to As through drinking of As-contaminated water and consumption of As-contaminated crops. The contaminated groundwater used to cultivate vegetables and rice for human consumption may be an important pathway of As ingestion and exposure to chronic As (Chakraborti et al, 2004). Food imported into the United Kingdom from Bangladesh has a 2- to 100-fold higher As concentration than vegetables cultivated in the United Kingdom and North America (Al Rmalli et al, 2005). In the South Punjab and Sindh provinces of Pakistan, As is a major contaminant of soil, water and fodder, thereby making its way into the food chain of cattle and subsequently the humans (Zubair et al, 2017). Thus, food is an important route of As exposure in some regions and these exposures can have long-term negative health effects in humans. Even, raised air-As concentration, caused by smelter contaminants in Yunnan (China) poisoning incident in 1993, has been found to contaminate crops grown in the area, where 90% of the human As intake is from the food (rice and corn), and only 10% or less from direct inhalation (Mandal and Suzuki, 2002).

The World Health Organization (WHO) certified that the maximum contaminant level (MCL) of As in drinking water is 10 μg/L (reduced from 50 μg/L) in 1993. Regulations in different countries have recommended different values of the MCL (Smedley, 2006; Barringer and Reilly, 2013). Countries like USA, New Zealand, Japan, Argentina, Brazil, Chile and Colombia place 10 μg/L as MCL in drinking water. Australia uses a standard of 7 μg/L. The New Jersey state in USA places 5 μg/L as the MCL. Mexico has adopted a standard of 25 μg/L, whereas some developing countries like Bangladesh have maintained the earlier 50 μg/L MCL standard. In July 2014, the WHO set worldwide guidelines for what it considers to be safe levels of As in rice, suggesting the maximum of 200 μg/kg for white rice and 400 μg/kg for brown rice (Sohn, 2014). Safety measures can be taken for removing As from drinking water (Ghosh and Singh, 2009) by various methods even at household levels (Chaurasia et al, 2012). However, removal of As from food or crop fields after its incorporation is not economically viable.

As is considered to be one of the most highly toxic and carcinogenic elements. According to the US Environmental Protection Agency (EPA) and the International Agency for Research on Cancer (IARC), As and its compounds have been ranked as a Group 1 human carcinogen. As present in drinking water or contaminated crops can have severe impact on the health of human beings as well as other animals (Ng et al, 2003). It has been found to be a fatal carcinogenic element in cases of extreme intake. Several deaths have been reported from areas of high contamination. As is a leading cause of serious health problems such as cancers of the skin, lung, bladder, liver and kidney, as well as adverse effects on cardiovascular, neurological, haematological, renal and respiratory systems (Barringer and Reilly, 2013).

As being taken up from soils, plant roots are the initial tissues to be exposed. The metalloid inhibits the extension and proliferation of roots. Upon its translocation to the shoot, As can inhibit plant growth severely by hindering the expansion and biomass accumulation as well as compromising the plant reproductive capacity through losses in fertility and yield (Rahman et al, 2007, 2008). At sufficiently high concentrations, As interferes with critical physiological and metabolic processes such as cellular membrane damage, and increase in antioxidant mechanisms, which can even lead to the death of the plant (Garg and Singla, 2011).

Occurrence and distribution of arsenic in rice growing areas

Rice, the dietary staple food for half of the world's population, is believed to have originated in Asia (Lahkar and Tanti, 2017), and gradually spread to all the continents. It has an affinity of taking up As from contaminated soil and water and even air to some extent (Abedin et al, 2002; Mandal and Suzuki, 2002). Thus, over the past few decades, with the increase in As content of soil and groundwater, and with the use of contaminated water in irrigation, subsequently, As content in rice grains has also been found to be rising in several parts of the world (Fig. 1).

The rice fields of Bangladesh are heavily irrigated with As contaminated water obtained from millions of shallow tube wells installed by the country's farmers. As a result, in several areas of the country, As content in rice grain has been found to rise to alarming levels which bears threat to food safety and also crop production in future (Williams et al, 2006; Rahman and Hasegawa, 2011). The highest mean As content in rice grains is found in Faridpur (0.95 μg/g), followed by Rajbari (0.76 μg/g), Golapganj (0.57 μg/g), Dinajpur (0.54 μg/g), Srinagar (0.48–0.53 μg/g) and Sonargaon (0.46 μg/g) districts of the country (Williams et al, 2006). Of the overall mean of 0.13 μg/g As in Bangladesh rice grains, about 80% is found to be inorganic, which is more toxic than the organic forms (Williams et al, 2006; Zavala et al, 2008).

In India, As-contaminated rice has been reported in the states of Chattisgarh (Patel et al, 2005) and West

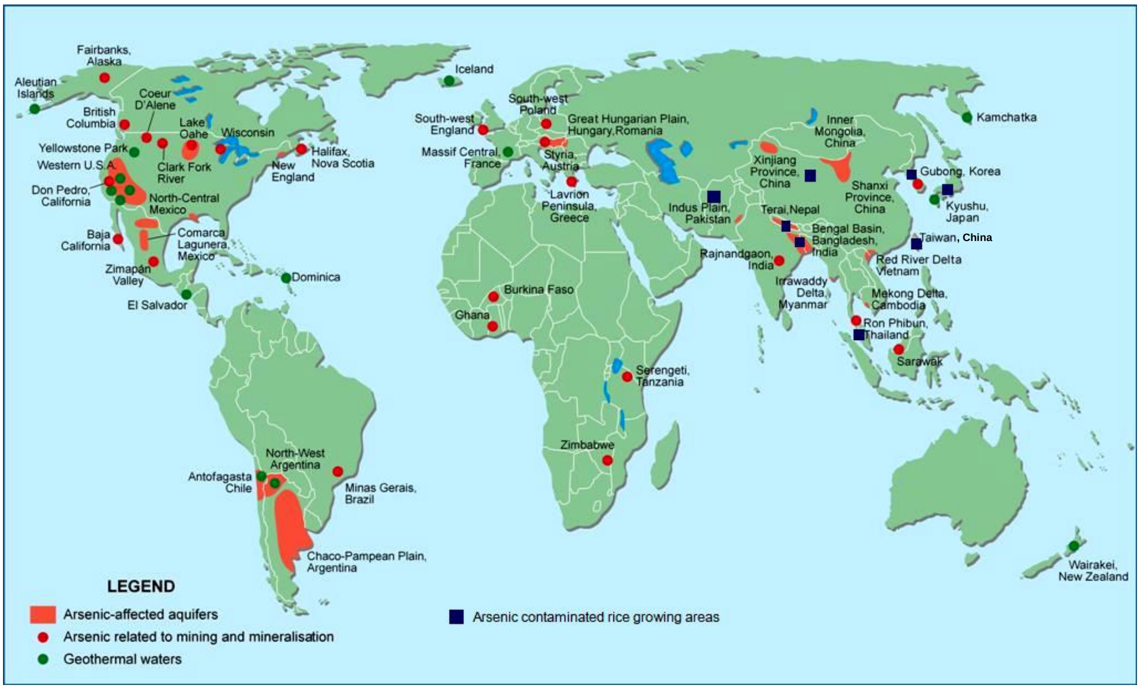

Fig. 1. Areas showing arsenic contaminated groundwater and rice fields.
Courtesy-British Geological Survey, Natural Environment Research Council (http://www.bgs.ac.uk/arsenic/).

Bengal (Santra et al, 2013), and more will potentially be reported in future, with groundwater contamination by As reported in a number of states (Singh, 2004; Chakraborti et al, 2012). In West Bengal, Boro rice varieties (0.451 μg/g) contain more As than Aman ones (0.334 μg/g), while improved high-yielding rice varieties also contain more As than the local ones (Bhattacharya et al, 2010b; Santra et al, 2013). Moreover, on dietary basis, daily As intake is 560 and 393 μg for adults and children, respectively, and the people having poor nutrition are affected more from As toxicity than those having adequate nutrition (Santra et al, 2013). In Chhattisgarh, the concentration of total As in rice samples is 0.018 to 0.446 μg/g (Patel et al, 2005).

In China, mining industries contribute a great deal to As contamination of rice, and the standard threshold for As concertration in food is 0.2 μg/g. As level in brown rice in Guangdong Province of China, which is susceptible to As poisoning from an abandoned tungsten mine (Lianhuashan), is 1.09 μg/g (Liu et al, 2010), which is several times higher than the national safety limit. The total As content in polished rice grains from different production regions of the country ranges from 0.065 to 0.274 μg/g, with an average value of 0.114 μg/g, where inorganic As is predominant, accounting for approximately 72% of the total As with a mean concentration of 0.082 μg/g (Liang et al, 2010). The major role (60%) in exposing the Chinese population to inorganic As is played by rice. Moreover, people from southern China are more prone to acquire cancer from As poisoning than those in the northern part of the country (Li et al, 2011).

Rice from USA contains higher total grain As content compared to rice from counties of Asia and Europe, although the amount of inorganic As (42% ± 5%) is lesser (Williams et al, 2006). Different reports suggest that rice from USA predominantly contains the organic form of As (Meharg et al, 2009), with DMA being a major species (Zavala et al, 2008). Besides these, presence of As in rice has also been reported in several other countries as mentioned in Table 1.

Uptake of arsenic by rice plants

As is available to rice plants in various forms from the soil water. Among the different species of As in soil, the concentration of one often dominates the rest depending upon the various physicochemical parameters in the soil environment. Inorganic As is dominant in soil water over organic (methylated) forms. Under oxidising conditions, the concentration of As^{5+} is the highest, while microbial activity may also lead to accumulation of MMA, DMA or TMAsO, whereas under reducing conditions, As^{3+} is the dominant form in soil (Mandal and Suzuki, 2002). Paddy is grown under waterlogging conditions, which is anaerobic and reducing in nature. The soil water in a paddy field goes on from being As^{5+} dominant to As^{3+} dominant with the passing of time during the cropping season as more and more irrigation water is provided. During the important stages of cropping from panicle development to grain filling, As content of soil water remains high, with the major species being As^{3+}, which is the more toxic form. Therefore, As^{3+} is the most important species of As in soil concerning rice crop growth and yield. Later on, as waterlogging conditions are over, near harvesting period, the soil conditions become oxidising and As^{5+} becomes the major form in the soil (Panaullah et al, 2008).

The uptake of the different species of As from the soil water by rice plants takes place at different rates. The amount of As taken up by rice plants followed the following trend: As^{3+} > MMA > As^{5+} > DMA (Marin et al, 1992). As^{3+} being very easily taken up by plants and also being the dominant one (Mandal and Suzuki, 2002) and one of the most phytotoxic forms (Marin et al, 1992), it is a major hazard for the rice growing and consuming communities of the world.

The transport rates of different As species within the rice plant vary considerably, which leads to variation in their accumulation in different organs of the plant (Fig. 2). Organic As species are more easily transported to the shoots in rice plants than the inorganic species (Marin et al, 1992; Raab et al, 2007; Carey et al, 2010). DMA is readily translocated to the

Table 1. Arsenic (As) found in sampled rice.

Country	Total As (μg/g)	Inorganic As (μg/g)	Reference
China [a]	0.19–0.76	0.11–0.51	Williams et al, 2006
Thailand	0.11 ± 0.01	0.08	Williams et al, 2006
Thailand	0.127–0.175	0.066–0.114	Torres-Escribano et al, 2008
Canada	0.02–0.11	0.01–0.08	Williams et al, 2006
France [a]	0.183	0.113	Torres-Escribano et al, 2008
Italy [a]	0.146–0.273	0.114–0.176	Torres-Escribano et al, 2008
Spain [a]	0.098–0.406	0.062–0.253	Torres-Escribano et al, 2008
Sweden [a]	0.20	0.11	Jorhem et al, 2007

[a] Samples were obtained from markets, and samples in China were from Taiwan Province.

shoots. However, the accumulation of the inorganic forms of As do not significantly increase with increase in As concentration in the growth medium (Marin et al, 1992). The majority of the As in rice grains is dominated by As^{3+} and DMA, and the former is retained only in the ovular vascular trace whereas the latter is dispersed throughout the external parts of the grain and up to the endosperm (Carey et al, 2010). This difference in As translocation and accumulation is prevalent in a number of other plant species belonging to several families of both monocots and dicots, suggesting a similarity in the basic mechanism of transport of the respective As species in different plants (Raab et al, 2007).

Different transporters are responsible for the uptake and translocation of different As species in the rice plants (Fig. 2). The uptake and translocation of As^{3+} are mediated by the rice silicic acid transporters Lsi1 (OsNIP2;1), belonging to nodulin26-like intrinsic protein (NIP) family of major intrinsic proteins and Lsi2 belonging to putative anion transporters (Ma et al, 2008). Lsi1 provides the ability to uptake As^{3+} from the medium, whereas Lsi2 helps in translocating As^{3+} to the shoots by regulating its efflux to the xylem. Transporters belonging to the plasma membrane intrinsic proteins (PIP) of rice, viz. OsPIP2;4, OsPIP2;6 and OsPIP2;7, are also observed to play a role in the transport of As^{3+} in heterologous systems (*Arabidopsis thaliana* and *Xenopus laevis* oocytes) by increasing membrane permeability. Among these transporters, OsPIP2;6 has also been demonstrated to efflux As^{3+} to the growth medium in transgenic *A. thaliana* overexpressing OsPIP2;6 during long term experiments, thus providing As^{3+} tolerance to the plant (Mosa et al, 2012). As^{5+} transport is regulated by phosphate transporters in several plants including rice. As^{5+} is an analogue of P, with its uptake being reduced by the presence of higher concentrations of P in the growth medium as determined in the As hyperaccumulator *Pteris vittata* (Wang et al, 2002), or *Brassica carinata* (Irtelli and Navari-Izzo, 2008). In case of rice, OsPHF1 (for phosphate transporter traffic facilitator 1) defective rice mutants uptake less Pi and As^{5+}. Moreover, transgenic rice overexpressing the Pi transporter OsPht1;8 (OsPT8) or those overexpressing the transcription factor OsPHR2 (for phosphate starvation response 2) uptake more Pi and As^{5+} in hydroponic culture, thus raising the maximum influx by three to five times (Carey et al, 2010) suggests strong affinity of Pi transporters for the uptake and translocation of As^{5+} in rice. Among the organic species of As, the methylated forms, MMA and DMA, are taken up by the rice aquaporin Lsi1 (Li et al, 2009).

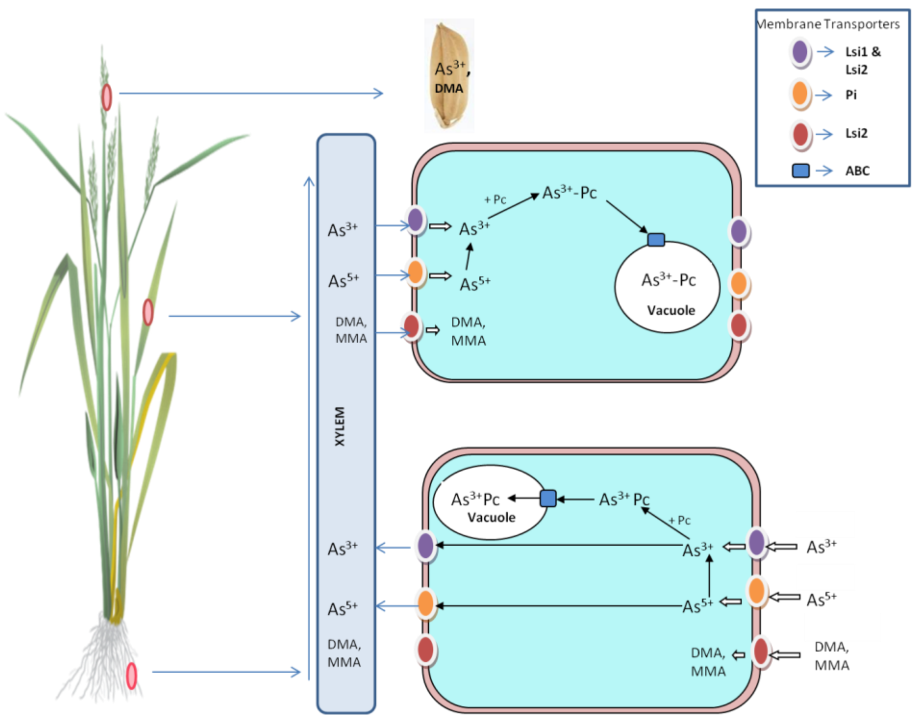

Fig. 2. Transport of arsenic (As) from roots to different parts of rice plants by means of various transporters.
DMA, Dimethyl arsenate; MMA, Monomethyl arsenate.

The translocation of DMA within the rice plant takes place at the highest rate among all the species of As (Marin et al, 1992). Along with As^{3+}, DMA forms the major part of As in the rice grains (Carey et al, 2010). However, in reports of As content in grains obtained from various affected countries, rice varieties varying in speciation of grain As content have been found, based on which two major groups of rice varieties can be identified, viz. one with more inorganic As in grains, and the other with more organic As in grains (Zavala et al, 2008). It has been recently shown that higher plants, including rice, are unable to methylate inorganic As (Lomax et al, 2012). However, there is evidence suggesting that organic As can be partially converted to inorganic forms by *Sinapis alba* (Jedynak et al, 2010). The exact mechanisms responsible for variation of the major As species in rice grains remain to be worked out.

Arsenic accumulation and toxicity

The amount of As accumulated in different parts of rice plants follows the order: straw > husk > grain (Abedin et al, 2002; Bhattacharya et al, 2010c). Abedin et al (2002) also showed that the concentrations of As in root and straw do not vary too much, suggesting that translocation from roots to straw takes place readily. However, its concentration decreases considerably in husk and yet further in grains.

From what evidence is present regarding transportation of different As species in rice plants, it can be seen that As^{3+} is the most easily taken up by roots, and DMA is the most easily translocated within the rice plants. As^{3+} and DMA are the two most prominent species of As that are translocated to the grains. However, a considerable amount of As^{5+} is also being taken up from the soil by the rice roots, and its concentration in the aboveground tissues of the plant is affected by mechanisms associated with As-tolerance response such as the presence of different arsenate reductases in rice, like OsHAC1;1, OsHAC1;2 and OsHAC4, which regulate the conversion of arsenate to arsenite. These genes are expressed mainly in the roots, and catalyse the reaction in the outer cell layer of root, thereby, facilitating arsenite efflux from the root to soil (Xu et al, 2017). A significant amount of As^{5+} is reduced to As^{3+} in root tissues of plants such as Indian mustard (Pickering et al, 2000), tomato and rice (Xu et al, 2007). Two ACR2-like genes, *OsACR2.1* and *OsACR2.2*, similar to the yeast *ACR2* genes responsible for arsenate reduction are identified in the rice genome, with the former being much active than the latter (Duan et al, 2007). Mutation of such genes led to decreased arsenate reduction in root, decreased arsenite efflux and increased As accumulation in root and grain. However, Chao et al (2014) demonstrated that *ACR2*-like genes are only found to reduce As^{5+} to As^{3+} *in vitro* and not in living plants. Moreover, the sequences of the yeast *ACR2* and *HAC1* genes found in plants are similar, and it is only the *HAC1* gene rather than the *ACR2*-like genes that takes part in arsenate reduction and arsenite efflux in plants. *HAC1* can therefore be an important candidate gene, which can reduce the accumulation of As in aboveground tissues due to its role in As^{3+} efflux mechanism.

Accumulation of As in rice varies according to the genotype of the plant (Norton et al, 2010) and its mobilization and intake is controlled by different factors such as As speciation, soil texture, pH and organic matter present (Mitra et al, 2017). The variation in accumulation may be due to the difference in presence of As in irrigation water, soils and the genetic makeup. The root characteristics also play a major role in As accumulation, which includes the root aeration, porosity (Wu et al, 2011), rhizosphere interactions (Dasgupta et al, 2004) and Fe-plaque formation on the root surfaces (Liu et al, 2004b). The solubility of As in the soils and its bioavailability to the rice plants is controlled by the soil texture and pH (Quazi et al, 2011). Silt and clayey soils with the fine texture provide large surface area for As scavenging compared to the sandy and loamy soils mainly based on the presence of Fe-oxides. Soil pH [both low (< 5.0) and high (> 8.5)] greatly affects As uptake and accumulation in the rice plants. The soils with low pH (< 5) provide higher As binding species such as Fe-oxyhydroxide compounds and enhance As uptake by plants. Under high pH, the negative surface charge is increased, giving rise to increase in hydroxyl ions, thus facilitating desorption of As from Fe-oxides and resulting in As transport in root vicinity and accumulation in the plant (Bhattacharya et al, 2010a; Ahmed et al, 2011). Organic matter has a greater affinity for As adsorption due to the formation of an organo-As complex, thereby reducing As availability to plants (Mitra et al, 2017).

Accumulation of As by rice plants results in various toxic reactions and affects the growth, morphological and physiological processes of the plant (Abbas et al, 2018). Increasing As content of growing medium or increased accumulation reduces chlorophyll content of the rice plants, the extent of reduction varying slightly among different varieties. This results in reduction in the rate of photosynthesis, leading to reduction in root and shoot growth and grain yield (Rahman et al, 2007; Halim et al, 2014). Yield reduction has also been observed with the increase in As content in soils in subsequent cropping years due to retention of As from previous years' contaminated irrigation water in the soils (Panaullah et al, 2008). A physiological disease called straight head is also attributed to As toxicity (Rahman et al, 2008). The symptoms of this disease include reduction in the sterility of florets/spikelets, desreased grain yield, and in severe cases, non-formation of the panicles or heads.

The level of sensitivity to As toxicity varies in different rice genotypes (Chaturvedi, 2013). Moreover, all the As species cause dissimilar levels of toxicity in rice plants. DMA is the least toxic form of As as determined by its effect on plant dry mass, and its application in low concentrations has only resulted in increased dry mass, while high concentration leads to no changes at all. Application of As^{3+} causes no significant change in dry mass. However, As^{3+} and MMA are highly toxic to plants, with the effect of MMA being greater than As^{3+} (Marin et al, 1992).

Arsenic hyperaccumulation and tolerance

It is well established that As accumulation leads to various toxic reactions in plants. However, some plants are tolerant to elevated levels of As and accumulate high levels of the metalloid in their bodies. The fern *Pteris vittata* is the first reported As hyperaccumulator whose fronds contain high amounts of mostly the toxic inorganic forms of As (Ma et al, 2001). *Sarcosphaera coronaria*, an ectomycorrhizal ascomycetous fungus, also accumulates very high amounts of As (mostly in the form of methylarsonic acid) in its fruit bodies (Falandysz and Borovička, 2013). The yeast ACR3 arsenite effluxer provides As tolerance on its heterologous expression in *Arabidopsis thaliana* (Ali et al, 2012). Two genes from *P. vittata*, *PvACR3* and *PvACR3;1*, similar to the yeast ACR3 arsenite effluxer, have been isolated. Of these, *PvACR3* has a definite role in As tolerance in the plant (Indriolo et al, 2010). ACR3 is a membrane protein. Once taken up by the roots, As^{5+} gets reduced to As^{3+}. *PvACR3* effluxes As^{3+} into the vacuole for sequestration. *ACR3* gene is absent in angiosperms. However, when *PvACR3* is expressed in transgenic *A. thaliana*, its tolerance to As is increased. *PvACR3* transgenic seeds of *A. thaliana* could even germinate in very high concentrations of As that are potentially lethal for wild type plants (Chen et al, 2013). *P. vittata* has been demonstrated to help in phytoremediation by removing As from soil. Rice cultivated in soils where *P. vittata* is grown previously has been found to accumulate less As in straw and grains (Ye et al, 2011).

QTLs for As accumulation in shoots have been identified in a doubled haploid population obtained from anther culture of F_1 plants generated from a cross between rice cultivars CJ06 (*japonica*) and TN1 (*indica*). The QTLs, *AsS*, *AsSe1* and *AsSe2*, are located on chromosomes 2, 6 and 8, respectively (Zhang et al, 2008). *AsSe2* collocates with the QTL for P accumulation in shoots at the seedling stage, having LOD value of 4.97.

Dasgupta et al (2004) found that in general *indica* rice varieties are more tolerant to As stress than *japonica* ones. In their experiments, CO39 is the most tolerant whereas Dawn is the most susceptible. They identified an As tolerance gene *AsTol* on chromosome 6 between markers RZ516 and RG213. Three tolerance genes in the rice genome play a part in As susceptibility or tolerance, and two are located on chromosome 6 whereas the other on chromosome 10. The genes, *AsTol6.1*, *AsTol6.2* and *AsTol10.1*, are epistatic in action with tolerance resulting in plants having inherited a minimum of two of the three genes from a tolerant parent (Norton et al, 2008). Plasma membrane intrinsic proteins from rice, OsPIP2;4, OsPIP2;6 and OsPIP2;7, when overexpressed in transgenic *Arabidopsis*, are found to increase As^{3+} tolerance in the plants during short term exposure (Mosa et al, 2012). These transporters might as well play important roles in rice tolerance to As. *Arabidopsis* plants mutant for auxin transporters have been found to be more sensitive to As^{3+} stress. Providing exogenous auxin improves As^{3+} tolerance in such mutants (Krishnamurthy and Rathinasabapathi, 2013). This hints at a possible role of auxin in ameliorating As stress in rice, however, the molecular basis of the mechanism need to be tested.

Phytochelatins in arsenic metabolism

Phytochelatins (PC) are thiol-rich peptides having the general formula γ(Glu-Cys)$_n$-Gly, where *n* ranges from 2 to 11 (called accordingly as PC_2, PC_3, PC_4, etc.), produced in plants in response to stress from heavy metals and metalloids including As (Hartley-Whitaker et al, 2001a). These are synthesized from glutathione by a specific y-glutamylcysteine dipeptidyl transpeptidase known as phytochelatin synthase (PCS) (Grill et al, 1989). PCs help in As detoxification by sequestrating it in the vacuoles. PCs bind As^{3+}, forming As-PC complexes which are then transported to vacuoles (Verbruggen et al, 2009). As^{5+} in plants is also reduced to As^{3+} (Pickering et al, 2000; Xu et al, 2007), which can be subsequently bound by PC and transported to the vacuoles. The transport of As-PC complexes to the vacuoles is mediated by the AtABCC1 and AtABCC2 transporters in *A. thaliana* (Song et al, 2010). Such ABC type transporters are supposed to help in transport of As-PC complexes in different plant species.

PC production in response to As stress has been demonstrated in plants like *Holcus lanatus*, *Agropyron repens*, *Glecoma hederacea*, *Leonurus marrubi-Astrum*, *Lolium perenne*, *Urtica dioica* and *Zea mays* (Hartley-Whitaker et al, 2001a, b; Schulz et al, 2007). In *A. thaliana*, overexpressing the phytochelatin synthase AtPCS1, tolerance to As stress, is increased (Li et al, 2004). In *H. lanatus*, concentration of PC increases with increasing As concentration in medium in case of tolerant and partially tolerant clones. However, in non-tolerant clones, PC production decreases at higher As^{5+} concentration in medium (Hartley-Whitaker et al, 2001a, b). The findings of Schulz et al (2007) and Hartley-Whitaker et al (2001b) agree on the dominant forms of PC in tolerant and non-tolerant plants. The dominant form of PC found in tolerant plants is PC_2 while in the non-tolerant plants it is PC_3. In intermediate tolerant clones of *H. lanatus*, PC_2 and PC_3 concentrations are approximately equal, while PC_4 is in lesser amounts in all clones. However, Schulz et al (2007) and Srivastava et al (2007) have shown that not all As is bound by PCs. After 4 d of treatment, the amount of As chelates by PCs in As^{3+} treated plants is 39%, while in As^{5+} treated plants, it is 35% (Srivastava et al, 2007). In the As hyperaccumulator fern *P. vittata*, only PC_2 is detected on As treatment. The concentration of PCs correlates significantly with As concentration. However, only a small portion of the As (1%–3%) is chelated with the PCs (Zhao et al, 2003). This suggest that PCs have a limited role in the tolerance of this fern to As stress.

In rice plants exposed to As stress, PCs belonging to the groups: PC [γ(Glu-Cys)$_n$-Gly, *n* ranges from 2 to 4], Ser-PCs [γ(Glu-Cys)$_n$-Ser, *n* ranges from 2 to 4], Des-PCs [γ(Glu-Cys)$_n$, *n* ranges from 2 to 3] and Glu-PCs, [γ(Glu-Cys)$_n$-Glu, *n* ranges from 2 to 3] are produced. The concentration of PCs correlates with the concentration of i-As in the roots (Batista et al, 2014). As-PC complexation in rice leaves leads to reduction in the translocation of As^{3+} to grains (Duan et al, 2007). However, the role of As-PC complexation in reducing grain As content is specific to certain varieties, while in others, different mechanisms such as As-PC release and transport seem to be decisive (Batista et al, 2014).

Oxidative response to arsenic stress

Intake of toxic metals leads to synthesis of reactive oxygen species (ROS) such as H_2O_2 in plants. These ROS cause oxidative injury in plants (Sarma et al, 2016), and plants resist this injury by various antioxidant metabolism reactions (Chutia et al, 2012). Plants may be tolerant or sensitive to metal toxicity. Tolerant plants often demonstrate a much competent antioxidant defence system than sensitive ones (Fig. 3).

As stress has also been found to induce oxidative response in plants. Polyamines involved in protection of plant structures against damage by ROS have been detected in red clover shoots after As treatment (Mascher et al, 2002). Moreover, the activities of superoxide dismutase (SOD) and peroxidase (POD) are detected in As-treated red clover shoots. The presence of three different SOD isoenzymes, Mn-SOD, Cu/Zn-SOD I and Cu/Zn-SOD II, have been determined. There is a tendency of antioxidant activity to increase with increasing concentration of As in culture when As concentration is low. However, their activities get inhibited at high metal concentration (Mascher et al,

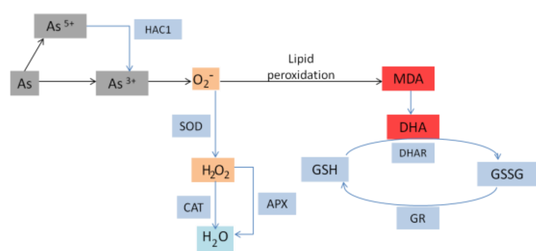

Fig. 3. Roles of antioxidants SOD, CAT, APX, GSH and GR in plants response to arsenic stress induced reactive oxygen species formation.

As, Arsenic; SOD, Superoxide dismutase; CAT, Catalase; APX, Ascorbate peroxidase; GSH, Glutathione; GR, Glutathione reductase; GSSG, Glutathione disulphide; MDA, Malondialdehyde; DHA, Dehydroascorbate; DHAR, Dehydroascorbate reductase; HAC1, High As content 1.

2002). Malondialdehyde (MDA) is an indicator of the occurrence of lipid peroxidation due to oxidative damage, which is detected in spinach leaves when grown in presence of As (Srivastava and Sharma, 2013; Hore et al, 2017; Nahar et al, 2018). Variation in the level of antioxidant activity is detected on comparison of tolerant and susceptible plant species. The As hyperaccumulator *Pteris vittata*, which is tolerant to much higher concentration of As, shows better resistance to oxidative injury than the non-hyperaccumulator *Pteris ensiformis*. *P. vittata* shows much less H_2O_2 concentration and lipid peroxidation as indicated by thiobarbituric acid-reactive substances (TBARS) formation as compared to *P. ensiformis*. Moreover, antioxidant defence is more competent in *P. vittata* than in *P. ensiformis* as indicated by ascorbate and glutathione pools. The ratios of reduced to oxidized forms of both ascorbate (AsA/DAsA) and glutathione (GSH/GSSG) are higher in *P. vittata* than in *P. ensiformis* under all treatments (Singh et al, 2006). In comparison of *Pisum sativum* and *Pennisetum typhoides*, it has been found that H_2O_2 concentration and lipid peroxidation are much higher in *P. sativum* than in *P. typhoides*. Moreover, concentrations of thiols, SOD activity, catalase (CAT) activity and ascorbate peroxidase (APX) activity indicate a much better antioxidant defence mechanism in *P. typhoides* than in *P. sativum*. This suggests that *P. typhoides* is the tolerant species whereas *P. sativum* is the sensitive one in response to As induced oxidative stress (Sharma, 2013). In tolerant plants, antioxidant activity is enhanced at high metal concentration and with the passing of time. However, it has been observed that, although antioxidant activity is enhanced with increasing metal accumulation initially, it gradually gets inhibited after a few days and the plants succumb to oxidative damage.

In rice, H_2O_2 concentration and lipid peroxidation level as indicated by MDA concentration increase with the accumulation of As. However, iron supplemented with As in the growth medium results in decrease of ROS concentration in the rice plants. Antioxidants CAT, SOD, AsA and glutathione (GSH) levels are also considerably high in only As^{5+} treated rice plants as compared to Fe supplemented ones (Nath et al, 2014). Fe plaque formation on root surface acts as a buffer, leading to the lowering of As translocation to shoots (Liu et al, 2004a, b). Thus, reducing As stress leads to reduction of ROS concentration and levels of antioxidants in rice plants. This suggests the activity of an antioxidant defence mechanism against As stress in rice plants. The variation of this defence mechanism in different varieties of rice plants need to be worked out in order to identify varieties tolerant to As in the growth medium.

Selenium (Se) has an ameliorating effect on As-induced oxidative stress measured in terms of H_2O_2 concentration, lipoxygenase (LOX) activity, lipid peroxidation as determined by estimation of MDA, and ion leakage measured in terms of electrical conductivity. Oxidative damage is found to decrease on supplementation of Se with As in the medium. The activities of CAT, APX and glutathione peroxidase (GPX) decrease under As treatment, but increase when As in the medium is supplemented with Se, indicating that Se boosts antioxidant activities and prevents any damage from oxidative stress (Kumar et al, 2015).

Role of rhizosphere microorganisms in arsenic stress

Soil rhizospheric microbial activity can affect As adsorption/desorption, solubility, bioavailability, mobility, and soil-plant transfer by altering the chemical speciation of As in soil (Ayangbenro and Babalola, 2017). Microorganisms can interconvert As^{3+} and As^{5+} and thus are capable of either solubilizing or immobilizing As in the soil-plant system (Suhadolnik et al, 2017). These microbially induced biotransformations of As play important roles in the biogeochemical behavior of As and are key in risk assessment and remediation studies (Anguita et al, 2017).

Arbuscular mycorrhizal fungi (AMF) associations can enhance resistance to As stress in plants. AMF such as *Glomus mosseae* and *G. caledonium* have been shown to reduce the uptake of As^{5+} by host *Holcus lanatus* plants, which are resistant to As (Gonzalez-Chavez et al, 2002). Moreover, AMF from contaminated sites are resistant to As stress compared to those from non-contaminated sites. However, both resistant and non-resistant AMF reduce the uptake of As by host plants. In a number of non-resistant plants including soybean and maize, mycorrhizal associations have proven to be beneficial for the host plants in resisting stress due to As (Bai et al, 2008; Spagnoletti and Lavado, 2015; Nahar et al, 2016).

Other microbes in the rhizosphere soil such as some fungi and bacteria also play important roles in determining the level of As toxicity on plants. The ectomychorrizal fungus *Sarchosphaera coronaria* accumulates high levels of As in its fruiting bodies

(Falandysz and Borovička, 2013). Other microbes have the capacity to transform As species in the soil, with great potential for increasing plant tolerance to As stress, and playing roles in bioremediation of As-contaminated soil and groundwater. Many rhizospheric N_2 fixing symbiotic microbes (eg. rhizobacteria) tolerate high metal concentration (Jaiswal, 2011). NT-26 strain of *Agrobacterium* / Rhizobium branch of α-Proteobacteria, strains MNZ1 (homologous to Enterobacter sp.), strains MNZ4 and MNZ6 (both homologous to *Klebsiella pneumoniae*) have been reported to oxidize As^{3+} to As^{5+} (Santini et al, 2000; Abbas et al, 2014). In natural environments a variety of As^{3+} oxidizers exist some autotrophic and others heterotrophic. They may further be aerobic and anaerobic. Mechanisms of As^{3+} oxidization varies among different groups, as indicated by the variation in gene and amino acid sequences of the arsenite oxidases (Rhine et al, 2007). This variation can be useful in isolation of useful microbial species that confers As stress resistance to plants in a variety of environments. Of particular importance in paddy cultivation can be the three strains, DAO1 (*Azoarcus* sp.), DAO10 (*Sinorhizobium* sp.) and MLHE-1 (*Alkalilimnicola ehrlichei*), which can oxidize As^{3+} in anaerobic conditions similar to those in submerged paddy cultivation. Furthermore, there are also strains that can reduce As^{5+} to As^{3+} such as *Desulfovibrio* strain Ben-RA and *Desulfomicrobium* strain Ben-RB (Macy et al, 2000). Indigenous microbes found in As contaminated environments are capable of transforming As. Both As^{5+} reducing and As^{3+} oxidising microbes often coexist and are supposed to be common in As contaminated sites (Macy et al, 2000). As^{3+} oxidising bacteria are also found to be effective in improving the bioremediation of As^{3+} contaminated sites by *Pteris vittata*. These microbes can potentially reduce the toxic effect of As on the growth of plants. In case of rice plants, microbes capable of transforming As^{3+} in anaerobic environment might be useful in promoting plant growth in As contaminated sites.

Agronomic mitigation of arsenic accumulation in rice

Different agronomic methods may be adopted to mitigate the effects of As accumulation in rice, which includes soil aeration by water management and preventing the reduction of As; creating the condition that forms the precipitation of insoluble As in soil; and decreasing As uptake and translocation in rice plants by augmenting mineral nutrients in soils that competes with As uptake (Fig. 4).

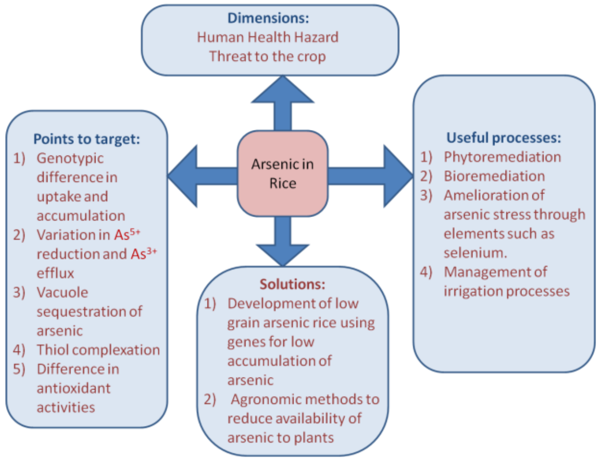

Fig. 4. Aspects of arsenic (As) stress in rice, and possible ways to deal with the problem.

Supplementation of soil with mineral nutrients (S, P, Fe and Si) can significantly decrease the accumulation of As in the food grains by minimizing its uptake and transport in food crops. Fe plaque plays an important role in diminution of As absorption around the roots of rice plants (Bakhat et al, 2017). P and Si which are complementary in nature to As competes for the intake by the rice plant through the transporter, thus the external application of P and Si reduces the chances of As intake from the soil.

Management of irrigation system or development of water saving mechanism maintains the redox level of soil and aggrandises the oxidation state that consequently hinders the reduction of As^{5+} to As^{3+}: which has prominently higher solubility, plant availability and toxicity (Takahashi et al, 2004). Thus, a water-saving regime has been reported to be an immediate and sustainable solution to decrease As content in rice (Arao et al, 2009). It has been reported that under aerobic water management practices, rice takes up less As (0.23–0.26 mg/kg) than under anaerobic practices (0.60–0.67 mg/kg) (Talukder et al, 2011).

Bioremediation strategies such as increase in number of soil microbes improved the mineral content of the soil through immobilization and mineralization thus affecting the availability and transport of As in the environment (Huang, 2014). Controlling the expression of transport mediator gene like *OsLsi1* and *OsLsi2* by AMF (Chen et al, 2012) also helps in mitigation of As toxicity.

CONCLUSIONS

The problem of As in rice has two dimensions, human health hazard due to incorporation of As in the food chain, and threat to the crop and sustainable agriculture due to the resulting stress.

Different transporters are involved in the uptake and translocation of As in rice plants. With the translocation rates of different As species varying with their respective transporters, rate of uptake and translocation of As in rice plants could be reduced by working out the underlying mechanisms. The cause of variation of the major As species in American and Asian rice need to be identified. The reason could be either genotypic, or due to difference in soil conditions and agricultural practices. If any difference at a molecular level exists between these varieties, it could be worked out to ensure incorporation of lesser inorganic As in rice grains. As efflux genes such as *HAC1* can be important in developing rice containing low grain As content.

Rice varieties have shown difference in tolerance to As, with the identification of mechanisms of tolerance such as As^{3+} efflux, vacuole sequestration or thiol complexation, and also genes for tolerance being detected, there is scope for traditional breeding approaches to develop tolerance to As among high yielding or traditional varieties. With phytohormone auxin being connected to As tolerance in *Arabidopsis* plants, such phenomenon in rice can be investigated. Hormone treatment of rice seedlings can be tried out to assess any change in tolerance to As stress.

Phytoremediation and land reclamation processes can make use of As-hyperaccumulators such as *Pteris vittata*. Bioremediation using As accumulating rhizosphere bacteria is also a feasible way to reduce the impact of As stress on rice. Elements that ameliorate the impact of As stress are useful when present in As contaminated soils. The formulation of optimum levels of these elements for extrogenous application, depending upon the soil type can alleviate As stress in rice plants. Moreover, management of irrigation is also important in limiting the soil arsenic levels, thereby reducing As uptake and accumulation in rice plants.

REFERENCES

Abbas S Z, Riaz M, Ramzan N, Zahid M T, Shakoori F R, Rafatullah M. 2014. Isolation and characterization of arsenic resistant bacteria from wastewater. *Braz J Microbiol*, **45**(4): 1309–1315.

Abbas G, Murtaza B, Bibi I, Shahid M, Niazi N K, Khan M I, Amjad M, Hussain M, Natasha. 2018. Arsenic uptake, toxicity, detoxification, and speciation in plants: Physiological, biochemical, and molecular aspects. *Int J Environ Res Public Health*, **15**(1): 59.

Abedin M J, Feldmann J, Meharg A A. 2002. Uptake kinetics of arsenic species in rice plants. *Plant Physiol*, **128**: 1120–1128.

Ahmed Z U, Panaullah G M, Gauch Jr H, McCouch S R, Ytagi W, Kabir M S, Duxbury J M. 2011. Genotype and environment effect rice (*Oryza sativa* L.) grain arsenic concentration in Bangladesh. *Plant Soil*, **338**: 367–382.

Al Rmalli S W, Haris P I, Harrington C F, Ayub M. 2005. A survey of arsenic in foodstuffs on sale in the United Kingdom and imported from Bangladesh. *Sci Total Environ*, **337**: 23–30.

Ali W, Isner J C, Isayenkov S V, Liu W, Zhao F J, Maathuis F J M. 2012. Heterologous expression of the yeast arsenite efflux system ACR3 improves *Arabidopsis thaliana* tolerance to arsenic stress. *New Phytol*, **194**(3): 716–723.

Anguita J M, Rojas C, Pastén P A, Vargas I T. 2017. A new aerobic chemolithoautotrophic arsenic oxidizing microorganism isolated from a high Andean watershed. *Biodegradation*, **29**(1): 59–69.

Arao T, Kawasaki A, Baba K, Mori S, Matsumoto S. 2009. Effects of water management on cadmium and arsenic accumulation and dimethylarsinic acid concentrations in Japanese rice. *Environ Sci Technol*, **43**: 9361–9367.

Ayangbenro A S, Babalola O O. 2017. A new strategy for heavy metal polluted environments: A review of microbial biosorbents. *Int J Environ Res Public Health*, **14**(1): 94.

Bai J F, Lin X G, Yin R, Zhang H Y, Wang J H, Chen X M, Luo Y M. 2008. The influence of arbuscular mycorrhizal fungi on As and P uptake by maize (*Zea mays* L.) from As-contaminated soils. *Appl Soil Ecol*, **38**(2): 137–145.

Bakhat H F, Zia Z, Fahad S, Abbas S, Hammad H M, Shahzad A N, Abbas F, Alharby H, Shahid M. 2017. Arsenic uptake, accumulation and toxicity in rice plants: Possible remedies for its detoxification: A review. *Environ Sci Pollut Res*, **24**(10): 9142–9158.

Barringer J L, Reilly P A. 2013. Arsenic in groundwater: A summary of sources and the biogeochemical and hydrogeologic factors affecting As occurrence and mobility. *In*: Current Perspectives in Contaminant Hydrology and Water Resources Sustainability. IntechOpen: 4.

Batista B L, Nigar M, Mestrot A, Rocha B A, Júnior F B, Price A H, Raab A, Feldmann J. 2014. Identification and quantification of phytochelatins in roots of rice to long-term exposure: Evidence of individual role on As accumulation and translocation. *J Exp Bot*, **65**(6): 1467–1479.

Bentley R, Chasteen T G. 2002. Microbial methylation of metalloids: Arsenic, antimony, and bismuth. *Microbiol Mol Biol Rev*, **66**(2): 250–271.

Bhattacharya P, Samal A C, Majumdar J, Santra S C. 2010a. Accumulation of arsenic and its distribution in rice plant (*Oryza* *sativa* L.) in gangetic West Bengal, India. *Paddy Water Environ*, **8**(1): 63–70.

Bhattacharya P, Samal A C, Majumdar J, Santra S C. 2010b. Arsenic contamination in rice, wheat, pulses, and vegetables: A study in an arsenic affected area of West Bengal, India. *Water Air Soil Pollut*, **213**(1): 3–13.

Bhattacharya P, Samal A C, Majumdar J, Santra S C. 2010c. Uptake of arsenic in rice plant varieties cultivated with arsenic rich groundwater. *Environ Asia*, **3**(2): 34–37.

Brammer H, Ravenscroft P. 2009. Arsenic in groundwater: A threat to sustainable agriculture in South and South-east Asia. *Environ Int*, **35**(3): 647–654.

Carey A M, Scheckel K G, Lombi E, Newville M, Choi Y, Norton G J, Charnock J M, Feldmann J, Price A H, Meharg A A. 2010. Grain unloading of arsenic species in rice. *Plant Physiol*, **152**(1): 309–319.

Chakraborti D, Sengupta M K, Rahman M M, Ahamed S, Chowdhury U K, Hossain M A, Mukherjee S C, Pati S, Saha K C, Dutta R N, Quamruzzaman Q. 2004. Groundwater arsenic contamination and its health effects in the Ganga-Meghna-Brahmaputra plain. *J Environ Monit*, **6**(6): 74–83.

Chakraborti D, Singh E J, Das B, Shah B A, Hossain M A, Nayak B, Ahamed S, Singh N R. 2012. Groundwater arsenic contamination in Manipur, one of the seven North-Eastern Hill states of India: A future danger. *Environ Earth Sci*, **56**(2): 381–390.

Chao D Y, Chen Y, Chen J G, Shi S L, Chen Z R, Wang C C, Danku J M, Zhao F J, Salt D E. 2014. Genome-wide association mapping identifies a new arsenate reductase enzyme critical for limiting arsenic accumulation in plants. *PLoS Biol*, **12**(12): e1002009.

Chaturvedi I. 2013. Genotypic differences in effects of arsenic on growth, and concentration of As in rice (*Oryza sativa*) genotypes. *Res J Recent Sci*, **2**(3): 49–52.

Chaurasia S C, Sahayam A C, Venkateswarlu G, Dhavile S M, Thangavel S, Rastogi L. 2012. Groundwater contamination problems in rural India: Detection and remediation at the household level. *BARC Newsl*, **324**: 39–45.

Chen X W, Li H, Chan W F, Wu C, Wu F Y, Wu S C, Wong M H. 2012. Arsenite transporters expression in rice (*Oryza sativa* L.) associated with arbuscular mycorrhizal fungi (AMF) colonization under different levels of arsenite stress. *Chemosphere*, **89**(10): 1248–1254.

Chen Y S, Xu W Z, Shen H L, Yan H L, Xu W X, He Z Y, Ma M. 2013. Engineering As tolerance and hyperaccumulation in plants for phytoremediation by a PvACR3 transgenic approach. *Environ Sci Technol*, **47**(16): 9355–9362.

Chutia J, Borah S P, Tanti B. 2012. Effect of drought stress on protein and proline metabolism in seven traditional rice (*Oryza sativa* Linn.) genotypes of Assam, India. *J Res Biol*, **2**(3): 206–214.

Dasgupta T, Hossain S A, Meharg A A, Price A H. 2004. An arsenate tolerance gene on chromosome 6 of rice. *New Phytol*, **163**(1): 45–49.

Duan G L, Zhou Y, Tong Y P, Mukhopadhyay R, Rosen B P, Zhu Y G. 2007. A CDC25 homologue from rice functions as an arsenate reductase. *New Phytol*, **174**(2): 311–321.

Erban L E, Gorelick S M, Zebker H A, Fendorf S. 2013. Release of arsenic to deep groundwater in the Mekong Delta, Vietnam, linked to pumping-induced land subsidence. *Proc Natl Acad Sci USA*, **110**(34): 13751–13756.

Falandysz J, Borovička J. 2013. Macro and trace mineral constituents and radionuclides in mushrooms: Health benefits and risks. *Appl Microbiol Biotechnol*, **97**(2): 477–501.

Garg N, Singla P. 2011. Arsenic toxicity in crop plants: Physiological effects and tolerance mechanisms. *Environ Chem Lett*, **9**(3): 303–321.

Garnier J M, Travassac F, Lenoble V, Rose J, Zheng Y, Hossain M S, Chowdhury S H, Biswas A K, Ahmed K M, Cheng Z, van Geen A. 2010. Temporal variations in arsenic uptake by rice plants in Bangladesh: The role of iron plaque in paddy fields irrigated with groundwater. *Sci Total Environ*, **408**(19): 4185–4193.

Ghosh N C, Singh R D. 2009. Groundwater Arsenic Contamination in India: Vulnerability and Scope for Remedy. Roorkee, Uttarakhand, India: National Institute of Hydrology.

Gonzalez-Chavez C, Harris P J, Dodd J, Meharg A A. 2002. Arbuscular mycorrhizal fungi confer enhanced arsenate resistance on *Holcus lanatus*. *New Phytol*, **155**(1): 163–171.

Grill E, Löffler S, Winnacker E L, Zenk M H. 1989. Phytochelatins, the heavy-metal-binding peptides of plants, are synthesized from glutathione by a specific *γ*-glutamylcysteine dipeptidyl transpeptidase (phytochelatin synthase). *Biochemistry*, **86**(18): 6838–6842.

Halim M A, Nigar M, Ghosh M, Akhter N, Hossain F. 2014. Interaction effects of arsenic and phosphorus on seedling growth, leaf pigments and leaf protein of rice cultivars under hydroponic culture. *J Asiat Soc Bangl*, **40**(1): 141–150.

Hartley-Whitaker J, Ainsworth G, Meharg A A. 2001a. Copper and arsenate-induced oxidative stress in *Holcus lanatus* L. clones with differential sensitivity. *Plant Cell Environ*, **24**(7): 713–722.

Hartley-Whitaker J, Ainsworth G, Vooijs R, Bookum W T, Schat H, Meharg A A. 2001b. Phytochelatins are involved in differential arsenate tolerance in *Holcus lanatus*. *Plant Physiol*, **126**(1): 299–306.

Hore P, Nahar S, Hasampura M, Tanti B. 2017. Phylogenetic analysis and homology modelling of betaine aldehyde dehydrogenase: An aroma producing protein in rice (*Oryza sativa* L.). *Int J Multidiscip Approach Stud*, **4**(3): 139–148.

Huang J H, Hu K N, Decker B. 2011. Organic arsenic in the soil environment: Speciation, occurrence, transformation, and adsorption behaviour. *Water Air Soil Pollut*, **219**: 401–415.

Huang J H. 2014. Impact of microorganisms on arsenic biogeochemistry: A review. *Water Air Soil Pollut*, **225**: 1848.

Indian Council of Agricultural Research (ICAR). 2009. Classification of pesticides based on their chemical nature. *Handbook Agric*, **6**: 716–719.

Indriolo E, Na G, Ellis D, Salt D E, Banks J A. 2010. A vacuolar arsenite transporter necessary for arsenic tolerance in the arsenic hyperaccumulating fern *Pteris vittata* is missing in flowering plants. *Plant Cell*, **22**: 2045–2057.

Irtelli B, Navari-Izzo F. 2008. Uptake kinetics of different arsenic species by *Brassica carinata*. *Plant Soil*, **303**: 105–113.

Jaiswal S. 2011. Role of rhizobacteria in reduction of arsenic uptake by plants: A review. *J Bioremed Biodegrad*, **2**: 4.

Jedynak L, Kowalska J, Kossykowska M, Golimowski J. 2010. Studies on the uptake of different arsenic forms and the influence of sample pretreatment on arsenic speciation in white mustard (*Sinapis alba*). *Microchem J*, **94**(2): 125–129.

Jorhem L, Astrand C, Sundström B, Baxter M, Stokes P, Lewis J, Grawé K P. 2007. Elements in rice on the Swedish market: 1. Cadmium, lead and arsenic (total and inorganic). *Food Add Contam: Part A*, **25**(3): 284–292.

Krishnamurthy A, Rathinasabapathi B. 2013. Auxin and its transport play a role in plant tolerance to arsenite-induced oxidative stress in *Arabidopsis thaliana*. *Plant Cell Environ*, **36**(10): 1838–1849.

Kumar S, Dubey R S, Tripathi R D, Chakrabarty D, Trivedi P K. 2015. Omics and biotechnology of arsenic stress and detoxification in plants: Current updates and prospective. *Environ Int*, **74**: 221–230.

Lahkar L, Tanti B. 2017. Study of morphological diversity of traditional aromatic rice landraces (*Oryza sativa* L.) collected from Assam, India. *Ann Plant Sci*, **6**(12): 1855–1861.

Li G, Sun G X, Williams P N, Nunes L, Zhu Y G. 2011. Inorganic arsenic in Chinese food and its cancer risk. *Environ Int*, **37**(7): 1219–1225.

Li R Y, Ago Y, Liu W J, Mitani N, Feldmann J, McGrath S P, Ma J F, Zhao F J. 2009. The rice aquaporin Lsi1 mediates uptake of methylated arsenic species. *Plant Physiol*, **150**(4): 2071–2080.

Li Y, Dhankher O P, Carreira L, Lee D, Chen A, Schroeder J I, Balish R S, Meagher R B. 2004. Overexpression of phytochelatin synthase in *Arabidopsis* leads to enhanced arsenic tolerance and cadmium hypersensitivity. *Plant Cell Physiol*, **45**(12): 1787–1797.

Liang F, Li Y, Zhang G L, Tan M G, Lin J, Liu W, Li Y, Lu W. 2010. Total and speciated arsenic levels in rice from China. *Food Addit Contam: Part A*, **27**(6): 810–816.

Liu C P, Luo C L, Gao Y, Li F B, Lin L W, Wu C A, Li X D. 2010. Arsenic contamination and potential health risk implications at an abandoned tungsten mine, southern China. *Environ Pollut*, **158**(3): 820–826.

Liu W J, Zhu Y G, Smith F A, Smith S E. 2004a. Do phosphorus nutrition and iron plaque alter arsenate (As) uptake by rice seedlings in hydroponic culture. *New Phytol*, **162**(2): 481–488.

Liu W J, Zhu Y G, Smith F A, Smith S E. 2004b. Do iron plaque and genotypes affect arsenate uptake and translocation by rice seedlings (*Oryza sativa* L.) grown in solution culture. *J Exp Bot*, **55**: 1707–1713.

Lomax C, Liu W J, Wu L Y, Xue K, Xiong J B, Zhou J Z, McGrath S P, Meharg A A, Miller A J, Zhao F J. 2012. Methylated arsenic species in plants originate from soil microorganisms. *New Phytol*, **193**(3): 665–672.

Ma J F, Yamaji N, Mitani N, Xu X Y, Su Y H, McGrath S P, Zhao F J. 2008. Transporters of arsenite in rice and their role in As accumulation in rice grain. *Proc Natl Acad Sci USA*, **105**(29): 9931–9935.

Ma L Q, Komar K M, Tu C, Zhang W, Cai Y, Kennelley E D. 2001. A fern that hyperaccumulates arsenic. *Nature*, **409**: 579.

Macy J M, Santini J M, Pauling B V, O'Neill A H, Sly L I. 2000. Two new arsenate/sulfate-reducing bacteria: Mechanisms of arsenate reduction. *Arch Microbiol*, **173**(1): 49–57.

Mandal B K, Suzuki K T. 2002. Arsenic round the world: A review. *Talanta*, **58**(1): 201–235.

Marin A R, Masscheleyn P H, Patrick Jr W H. 1992. The influence of chemical form and concentration of arsenic on rice growth and tissue arsenic concentration. *Plant Soil*, **139**(2): 175–183.

Mascher R, Lippmann B, Holzinger S, Bergmann H. 2002. Arsenate toxicity: Effects on oxidative stress response molecules and enzymes in red clover plants. *Plant Sci*, **163**(5): 961–969.

Meharg A A, Williams P N, Adomako E, Lawgali Y Y, Deacon C, Villada A, Cambell R C J, Sun G, Zhu Y G, Feldmann J, Raab A, Zhao F J, Islam R, Hossain S, Yanai J. 2009. Geographical variation in total and inorganic arsenic content of polished (white) rice. *Environ Sci Technol*, **43**(5): 1612–1617.

Mitra A, Chatterjee S, Moogouei R, Gupta D K. 2017. Arsenic accumulation in rice and probable mitigation approaches: A review. *Agronomy*, **7**(4): 67.

Mosa K A, Kumar K, Chhikara S, McDermott J, Liu Z, Musante C, White J C, Dhankher O P. 2012. Members of rice plasma membrane intrinsic proteins subfamily are involved in arsenite permeability and tolerance in plants. *Transg Res*, **21**(6): 1265–1277.

Nahar S, Kalita J, Sahoo L, Tanti B. 2016. Morphophysiological and molecular effects of drought stress in rice. *Ann Plant Sci*, **5**(9): 1409–1416.

Nahar S, Vemireddy L R, Sahoo L, Tanti B. 2018. Antioxidant protection mechanisms reveal significant response in drought-induced oxidative stress in some traditional rice of Assam, India. *Rice Sci*, **25**(4): 185–196.

Nath S, Panda P, Mishra S, Dey M, Choudhury S, Sahoo L, Panda S K. 2014. Arsenic stress in rice: Redox consequences and regulation by iron. *Plant Physiol Biochem*, **80**: 203–210.

Ng J C, Wang J P, Shraim A. 2003. A global health problem caused by arsenic from natural sources. *Chemosphere*, **52**(9): 1353–1359.

Norton G J, Nigar M, Williams P N, Dasgupta T, Meharg A A, Price A H. 2008. Rice-arsenate interactions in hydroponics: A three-gene model for tolerance. *J Exp Bot*, **59**(8): 2277–2284.

Norton G J, Deacon C M, Xiong L Z, Huang S Y, Meharg A A, Price A H. 2010. Genetic mapping of the rice ionome in leaves and grain: Identification of QTLs for 17 elements including arsenic, cadmium, iron and selenium. *Plant Soil*, **329**: 139–153.

Panaullah G M, Alam T, Hossain M B, Loeppert R H, Lauren J G, Meisner C A, Ahmed Z U, Duxbury J M. 2008. Arsenic toxicity to rice (*Oryza sativa* L.) in Bangladesh. *Plant Soil*, **317**(1): 31–39.

Patel K S, Shrivas K, Brandt R, Jakubowski N, Corns W, Hoffmann P. 2005. Arsenic contamination in water, soil, sediment and rice of central India. *Environ Geochem Health*, **27**(2): 131–145.

Pickering I J, Prince R C, George M J, Smith R D, George G N, Salt D E. 2000. Reduction and coordination of arsenic in Indian Mustard. *Plant Physiol*, **122**(4): 1171–1177.

Quazi S, Datta R, Sarkar D. 2011. Effect of soil types and forms of arsenical pesticide on rice growth and development. *Int J Environ Sci Technol*, **8**(3): 450–460.

Raab A, Williams P N, Meharg A, Feldmann J. 2007. Uptake and translocation of inorganic and methylated arsenic species by plants. *Environ Chem*, **4**: 197–203.

Rahman M A, Hasegawa H, Rahman M M, Islam M N, Miah M A M, Tasmin A. 2007. Effect of arsenic on photosynthesis, growth and yield of five widely cultivated rice (*Oryza sativa* L.) varieties in Bangladesh. *Chemosphere*, **67**(6): 1072–1079.

Rahman M A, Hasegawa H, Rahman M M, Miah M A M, Tasmin A. 2008. Straighthead disease of rice (*Oryza sativa* L.) induced by arsenic toxicity. *Environ Exp Bot*, **62**(1): 54–59.

Rahman M A, Hasegawa H. 2011. High levels of inorganic arsenic in rice in areas where arsenic contaminated water is used for irrigation and cooking. *Sci Total Environ*, **409**(22): 4645–4655.

Rhine E D, Ní Chadhain S M, Zylstra G J, Young L Y. 2007. The arsenite oxidase genes (aroAB) in novel chemoautotrophic arsenite oxidizers. *Biochem Biophys Res Commun*, **354**(3): 662–667.

Santini J M, Sly L I, Schnagl R D, Macy J M. 2000. A new chemolithoautotrophic arsenite-oxidizing bacterium isolated from a gold mine: Phylogenetic, physiological, and preliminary biochemical studies. *Appl Environ Microbiol*, **66**(1): 92–97.

Santra S C, Samal A C, Bhattacharya P, Banerjee S, Biswas A, Majumdar J. 2013. Arsenic in foodchain and community health risk: A study in Gangetic West Bengal. *Proced Environ Sci*, **18**: 2–13.

Sarma B, Basumatary N R, Nahar S, Tanti B. 2016. Effect of drought stress on morpho-physiological traits in some traditional rice cultivars of Kokrajhar district, Assam, India. *Ann Plant Sci*, **5**(8): 1402–1408.

Schulz H, Härtling S, Tanneberg H. 2007. The identification and quantification of arsenic-induced phytochelatins comparison between plants with varying arsenic sensitivities. *Plant Soil*, **303**: 275–287.

Sharma I. 2013. Arsenic-induced oxidative stress and antioxidant defense system of *Pisum sativum* and *Pennisetum typhoides*: A comparative study. *Res J Biotechnol*, **8**(4): 48–56.

Singh A K. 2004. Arsenic Contamination in Groundwater of North Eastern India. *In*: Proceedings of National seminar on Hydrology with focal theme on Water Quality. Roorkee, India: National Institute of Hydrology: 255–262.

Singh N, Ma L Q, Srivastava M, Rathinasabapathi B. 2006. Metabolic adaptations to arsenic-induced oxidative stress in *Pteris vittata* L. and *Pteris ensiformis* L. *Plant Sci*, **170**(2): 274–282.

Smedley P L, Kinniburgh D G. 2002. A review of the source, behaviour and distribution of arsenic in natural waters. *Appl Geochem*, **17**(5): 517–568.

Smedley P L. 2006. Sources and distribution of arsenic in groundwater and aquifers. *In*: Proceedings in Seminar: Arsenic in Groundwater: A World Problem. Utrecht.

Sohn E. 2014. Contamination: The toxic side of rice. *Nature*, **514**: 62–63.

Song W Y, Park J, Mendoza-Cózatl D G, Suter-Grotemeyer M, Shim D, Hörtensteiner S, Geisler M, Weder B, Rea P A, Rentsch D, Schroeder J I, Lee Y, Martinoia E. 2010. Arsenic tolerance in *Arabidopsis* is mediated by two ABCC-type phytochelatin transporters. *Proc Natl Acad Sci USA*, **107**: 21187–21192.

Spagnoletti F, Lavado R S. 2015. The arbuscular mycorrhiza rhizophagus intraradices reduces the negative effects of arsenic on soybean plants. *Agron J*, **5**(2): 188–199.

Srivastava S, Mishra S, Tripathi R D, Dwivedi S, Trivedi P K, Tandon P K. 2007. Phytochelatins and antioxidant systems respond differentially during arsenite and arsenate stress in *Hydrilla verticillata* (L.f.) Royle. *Environ Sci Technol*, **41**(8): 2930–2936.

Srivastava S, Sharma Y K. 2013. Altered growth, photosynthetic machinery and induced oxidative stress in spinach in response to arsenic stress. *J Plant Physiol Pathol*, **1**: 4.

Suhadolnik M L S, Salgado A P C, Scholte L L S, Bleicher L, Costa P S, Reis M P, Dias M F, Avila M P, Barbosa F A R, Chartone-Souza E, Nascimento A M A. 2017. Novel arsenic-transforming bacteria and the diversity of their arsenic-related genes and enzymes arising from arsenic-polluted freshwater sediment. *Sci Rep*, **7**(1): 1–17.

Takahashi Y, Minamikawa R, Hattori K H, Kurishima K, Kihou N, Yuita K. 2004. Arsenic behaviour in paddy fields during the cycle of flooded and non-flooded periods. *Environ Sci Technol*, **38**(4): 1038–1044.

Talukder A S M H M, Meisner C A, Sarkar M A R, Islam M S. 2011. Effect of water management, tillage options and phosphorus status on arsenic uptake in rice. *Ecotoxicol Environ Safety*, **74**(4): 834–839.

Torres-Escribano S, Leal M, Vélez D, Montoro R. 2008. Total and inorganic arsenic concentrations in rice sold in Spain, effect of cooking, and risk assessments. *Environ Sci Technol*, **42**(10): 3867–3872.

Turpeinen R, Pantsar-Kallio M, Kairesalo T. 2002. Role of microbes in controlling the speciation of arsenic and production of arsines in contaminated soils. *Sci Total Environ*, **285**: 133–145.

Verbruggen N, Hermans C, Schat H. 2009. Mechanisms to cope with arsenic or cadmium excess in plants. *Curr Opin Plant Biol*, **12**(3): 364–372.

Wang J R, Zhao F J, Meharg A A, Raab A, Feldmann, McGrath S P. 2002. Mechanisms of arsenic hyperaccumulation in *Pteris vittata*: Uptake kinetics, interactions with phosphate, and arsenic speciation. *Plant Physiol*, **130**(3): 1552–1561.

Williams P N, Price A H, Raab A, Hossain S A, Feldmann J, Meharg A A. 2005. Variation in arsenic speciation and concentration in paddy rice related to dietary exposure. *Environ Sci Technol*, **39**(15): 5531–5540.

Williams P N, Islam M R, Adomako E E, Raab A, Hossain S A, Zhu Y G, Feldman J, Meharg A A. 2006. Increase in rice grain arsenic for regions of Bangladesh irrigating paddies with elevated arsenic in groundwaters. *Environ Sci Technol*, **40**: 4903–4908.

Woolson E A. 1977. Fate of arsenicals in different environmental substrates. *Environ Health Perspect*, **19**: 73–81.

Wu Z C, Ren H Y, McGrath S P, Wu P, Zhao F J. 2011. Investigating the contribution of the phosphate transport pathway to arsenic accumulation in rice. *Plant Physiol*, **157**(1): 498–508.

Xu J M, Shi S L, Wang L, Tang Z, Lv T T, Zhu X L, Ding X M, Wang Y F, Zhao F J, Wu Z C. 2017. OsHAC4 is critical for arsenate tolerance and regulates arsenic accumulation in rice. *New Phytol*, **215**(3): 1090–1101.

Xu X Y, McGrath S P, Zhao F J. 2007. Rapid reduction of arsenate in the medium mediated by plant roots. *New Phytol*, **176**(3): 590–599.

Xu X Y, McGrath S P, Meharg A A, Zhao F J. 2008. Growing rice aerobically markedly decreases arsenic accumulation. *Environ Sci Technol*, **42**(15): 5574–5579.

Ye W L, Khan M A, McGrath S P, Zhao F J. 2011. Phytoremediation of arsenic contaminated paddy soils with *Pteris vittata* markedly reduces arsenic uptake by rice. *Environ Pollut*, **159**(12): 3739–3743.

Zavala Y J, Gerads R, Gürleyük H, Duxbury J M. 2008. Arsenic in Rice: II. Arsenic speciation in USA grain and implications for human health. *Environ Sci Technol*, **42**(10): 3861–3866.

Zhang J, Zhu Y G, Zeng D L, Cheng W D, Qian Q, Duan G L. 2008. Mapping quantitative trait loci associated with arsenic accumulation in rice (*Oryza sativa*). *New Phytol*, **177**(2): 350–355.

Zhao F J, Wang J R, Barker J H A, Schat H, Bleeker P M, McGrath S P. 2003. The role of phytochelatins in arsenic tolerance in the hyperaccumulator *Pteris vittata*. *New Phytol*, **159**(2): 403–410.

Zubair M, Martyniuk C J, Shaheen A. 2017. Rising level of arsenic in water and fodder: A growing threat to livestock and human populations in Pakistan. *Toxin Rev*: 1–11.

(Managing Editor: LI Guan)