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The Potential Use of *Vetiveria zizanioides* for the Phytoremediation of Antimony, Arsenic and Their Co-Contamination

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Abstract Antimony (Sb) and arsenic (As) contaminations are the well reported and alarming issues of various contaminated smelting and mining sites all over the world, especially in China. The present hydroponic study was to assess the capacity of *Vetiveria zizanioides* for Sb, As and their interactive accumulations. The novelty of the present research is this that the potential of *V. zizanioides* for Sb and As alone and their interactive accumulation are unaddressed. This is the first report about the interactive co-accumulation of Sb and As in *V. zizanioides*. Highest applied Sb and As contaminations significantly inhibited the plant growth. Applied Sb and As alone significantly increased their concentrations in the roots/shoot of *V. zizanioides*. While co-contamination of Sb and As steadily increased their concentrations, in the plant. The co-contamination of Sb and As revealed a positive correlation between the two, as they supplemented the uptake and accumulation of each other. The overall translocation (TF) and bioaccumulation factors (BF) of Sb in *V. zizanioides*, were 0.75 and 4. While the TF and BF of As in *V. zizanioides*, were 0.86 and 10. *V. zizanioides* proved as an effective choice for the phytoremediation and ecosystem restoration of Sb and As contaminated areas.

Keywords Vetiver (*Vetiveria zizanioides* L.) · Antimony (Sb) · Arsenic (As) · Co-contamination · Phytoremediation · Hydroponics

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Soil metal contamination due to mining activities became a major threat to land use. Different plant species could be used for the remediation of metal contaminated soils (Rizwan et al. 2017; Chai et al. 2017; Wang et al. 2016; Tauqeer et al. 2016; Khan et al. 2015). Antimony and arsenic are the hazardous and toxic metalloids which are commonly present in the environment. They are both naturally occurring analogs belong to the same group and share similar chemical properties. Their well reported contamination at and around mining sites of the world is believed to be the result of increasing mining and incineration (Danh et al. 2009; Okkenhaug et al. 2011; Ehsan et al. 2016). Natural and anthropogenic activities have resulted in the severe spread of Sb and/or As contamination of both the soil and ground water. Sulfide ores, comprised of high Sb are often accompanied with high As concentrations (Okkenhaug et al. 2011; Danh et al. 2009), which unavoidably results in their co-contaminations. Intensive accumulation and spread of Sb and As at some locations have been reported (Wang et al. 2015; Cidu et al. 2014; Feng et al. 2015;

Mubarak et al. 2016; Mirza et al. 2010). Arsenic pollution has become a major hazard to human health in many areas (Dziubinska et al. 2012; Martinez et al. 2011). Worldwide more than 40 million people are chronically exposed to the higher levels of As and suffering from numerous diseases including various types of cancers (Yang et al. 2009). On one hand they are the by-products of mining while on the other due to their cytotoxic properties they are being used for clinical treatments such as anticancer, acute promyelocytic leukemia, hematological malignancies, solid tumors (Dilda and Hogg 2007), leishmaniasis (Frézard and Demicheli 2010), sleeping sickness (Dziubinska et al. 2012) and antiprotozoan therapies (Dilda and Hogg 2007).

Both Sb and As are non-essential and toxic elements to the plants. However, tolerant plants can readily take up both the Sb and As to some extent (Mubarak et al. 2015; Corrales et al. 2014). On entering into the farming systems through natural geochemical processes, they contaminate crops and fodders hence become part of the food chain. The Sb and As metalloids inhibit root elongation and shoot growth. Upon translocation to the shoot, they can severely inhibit plant growth by slowing biomass accumulation, loss in fertility, reduction in yield and ceased fruit production. At severely high concentrations, they can interfere with metabolism which can lead to the death of the plant (Finnegan and Chen 2012; Tschan et al. 2009). Numerous physiological processes are susceptible to As and Sb toxicity. Their exposure induces oxidative stress by damaging cellular membranes, accompanied by increased malondialdehyde. Non-accumulators retain these toxicants in the roots, with much lower concentrations in the shoots. The growths of the hyper-accumulators do not get compromised even at high levels of accumulations; they possess high concentrations in the aerial parts as compared to the root (Finnegan and Chen 2012; Tschan et al. 2009). Hence, they are a matter of increased environmental concern (Okkenhaug et al. 2011, 2012). The removal of Sb and/or As from contaminated areas (mining areas) can be promoted by using tolerant plants to accumulate the contaminant, e.g., Sb and/or As (Couto et al. 2015; Vaculík et al. 2013).

Previous reports on Sb and As phytoremediation (plants, ferns, grasses), focused either of them alone. The main contents of the reports were about the translocation and accumulation of metals within the plant parts. (Tschan et al. 2009; Mirza et al. 2010; Feng et al. 2015; Chai et al. 2016; Mubarak et al. 2016). But the phytoremediation potential of the reported plants for Sb and As pollution is rarely documented. Investigation of the interactive effects of Sb and As on accumulations and distributions in tolerant plants may be helpful in understanding the potentials of the plants for the decontamination of Sb and As co-contamination. The effective removal of Sb and As from a contaminated site by phytoremediation involves tolerance of the plant, growth rate, properties of soil and the availability of contaminants (Couto et al. 2015).

Vetiveria zizanioides (L.) Nash, is a perennial tropical grass with stiff stems, dense and massive root system. It is high biomass producing, tolerant and adaptive to a wide range of climatic and environmental variations i.e., pH 3.3–9.5, saline, sodic, drought, water logg, heavy metal pollution (Chen et al. 2004). It is reported as an excellent—a choice plant for phytoremediation of heavy metal and organic wastes. As it has proved as a potential candidate for the phytoremediation of heavy metals from contaminated areas (Caporale et al. 2014; Danh et al. 2009), it is gaining interest of researchers for the remediation of a wide range of pollutants. The present study was carried out to investigate the effects of Sb and As alone and together on the plant. The potential of *V. zizanioides* for the uptake of Sb and As alone and together from the Sb and As contaminated hydroponics were also accessed.

Materials and Methods

Young plants of *V. zizanioides*, (25:15 cm shoot:root) containing leaves were collected from nursery and were grown in the fields of Central South University for 2 months. Later required plants were collected, thoroughly rinsed with tape water, followed by deionized water and were grown in a greenhouse in the half strength Hoagland solution (2 L) for acclimation. The illumination was maintained using three fluorescent lamps providing a 14/10 h (light/dark) cycle. Day/night temperatures and humidity were 25/18°C and 60%–80%, respectively. All potted plants were well-watered, for about 3 weeks.

After 3 weeks acclimation, the plants were transferred into 2 L of half strength Hoagland solution spiked with Sb, As and both. All the chemicals used in the research were of analytical reagent (AR) grade. Sb and As were applied as $KSbC_4H_4O_7 \cdot 1/2H_2O$ (Sb-III, >99% purity) and $NaAsO_2$ (As-III, >99% purity). The nutrient content of the Hoagland solution was as Wang et al. (2017) and Chai et al. (2016). Each treatment was replicated three times. Using a styrofoam plug, each plant was fixed and protected in a tray. The aeration in each pot was ensured using air pumps. The culture conditions of the treated pots were the same during the 3 weeks of acclimation.

After 21 days of Sb and As exposure, to remove the excess metal adsorbed to the root surface, the roots were immersed in 20 mM Disodium Ethylenediaminetetraacetic acid, Na_2-EDTA (30 min). The entire plants were rinsed three times with deionized water. The plant parts of shoot and roots were separated. For further analysis the larger part of the samples were dried at 70°C for 72 h. The remaining Hoagland solution was filtered and refrigerated for the Sb and As analysis. The phytotoxicity effect of Sb and As, and the stress tolerance index in Sb and As contaminated *V. zizanioides* were calculated according to the Chai et al. (2016).

The dried plant samples were ground, sieved (1 mm sieve) and digested with HNO_3:$HClO_4$ (4:1, v/v). For accuracy of the digestion and analytical method, a blank sample (4 mL HNO_3 + 1 mL $HClO_4$) was also run with the samples. Sb and As concentrations in the Hoagland solution and the plant parts were analyzed through Induce Couple Plasma—Optical Emission Spectrometer (ICP-OES) (Mubarak et al. 2016; Lee et al. 2013). For quality assurance (QA) and quality control (QC) for Sb and or As in *V. zizanioides*, a standard reference material (SRM) i.e., GBW07603, purchased from the National Information Center for Certified Reference Material of China was used. Thus, the accuracy of the elemental analysis were assessed using standard reference material (GBW-07603) purchased from the Center for Standard Reference of China. The bioaccumulation factor (BF) and translocation factor (TF) of Sb and As in *V. zizanioides* were calculated (Mirza et al. 2011) as the ratio of Sb and/or As concentration in the shoots to the Sb and/or As in solution and the Sb and/or As in the shoot to the Sb and/or As concentration in the roots, respectively, in *V. zizanioides*.

An analysis of variance (ANOVA) at a significance level of $\rho < 0.05$ was performed using the General Linear Model (GLM) in the SAS package. The LSD test and *t* test were employed to compare significant differences between the means for the treatments at $\rho < 0.05$. All the results are expressed as the means ± SD. Graphical analysis was carried out using Origin Pro 8.5.

Result and Discussion

Increasing Sb, As and Sb + As contaminations significantly ($\rho < 0.05$) inhibited the growth of *V. zizanioides* (Fig. 1a). As compared to control, the pronounced inhibition in the plant heights of Sb, As and Sb + As contaminated *V. zizanioides* was under low, moderate and high Sb + As co-contamination ($Sb_{10+}As_{10}$, $Sb_{20+}As_{20}$ and $Sb_{40+}As_{40}$ mg L^{-1}) after Sb alone (Sb_{10}, Sb_{20}, Sb_{40}, Sb_{80} mg L^{-1}) and least was under As alone (As_{10}, As_{20}, As_{40}, As_{80} mg L^{-1}). The decrease in the plant heights of *V. zizanioides* under Sb_{10}, Sb_{20}, Sb_{40}, and Sb_{80} mg L^{-1} were 6 ± 2.30, 9 ± 1.90, 12 ± 1.30 and 3 ± 1.56 cm (14%, 22%, 28% and 6% < control), respectively. As compared to control, at As_{10}, As_{20}, As_{40}, and As_{80} mg L^{-1}, the plant heights were reduced to 7 ± 1.00, 4 ± 1.00, 6 ± 0.85 and 9 ± 1.01 cm (15%, 9%, 15% and 22% < control), respectively (Fig. 1a). While, the reductions in the plant height of *V. zizanioides*, at low, moderate and high Sb + As, were 7.0 ± 2.5, 10 ± 1.5 and 30 ± 1.4 (17%, 24% and 30% < control) respectively (Fig. 1a). Throughout the experiment, the plants were green and healthy. The novelty of the present research is that

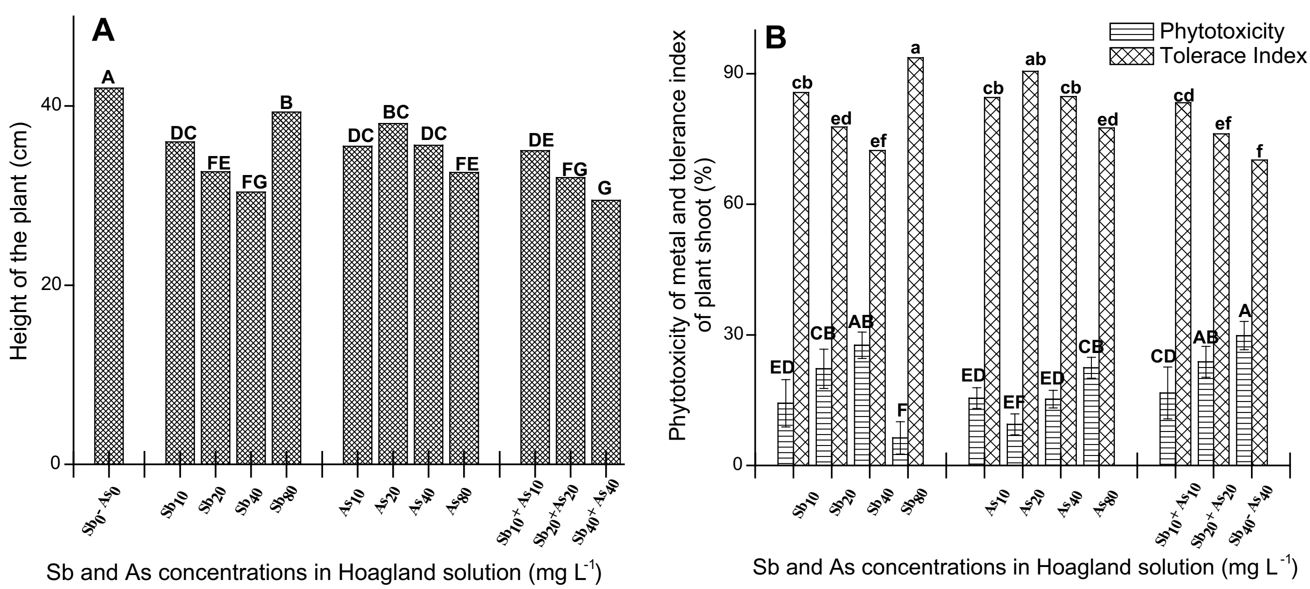

Fig. 1 **a** Effect of Sb and As application, on the plant height of *Vetiveria zizanioides*. **b** Phytotoxicity of Sb and As application and tolerance index of *V. zizanioides*. Values followed by *different uppercase letters* are significantly different at ρ < 0.05, for treatments. Values followed by *different lowercase letters* are significantly different at ρ < 0.05, for treatments. *Different letters* indicate significant differences between treatments for each parameter, at significant level of 0.05. Values followed by the same letters for each parameter are not significantly different at the 0.05 level (least significant difference). Values in the graph are mean (n = 3), *error bars* are standard deviation (SD)

previously, no study has yet reported the co-contamination of *V. zizanioides* by Sb and As. Secondly the effects of Sb and As co-contamination on the growth and accumulation of both the contaminants in *V. zizanioides* are unaddressed.

The phytotoxic effects of Sb and As on *V. zizanioides*, were significantly ($\rho < 0.05$), different. The phytotoxic effects of applied Sb, As and Sb + As on the *V. zizanioides* were between 6%–28%, 9%–22% and 17%–30%, respectively (Fig. 1b). The tolerance indices of *V. zizanioides*, with the increasing applied Sb, As and Sb + As, were significantly ($\rho < 0.05$), different. At applied Sb, As and Sb + As concentrations, the tolerance index (%) of *V. zizanioides* has revealed the resistance of the plant. The stress tolerance indices of Sb, As and Sb + As contaminated *V. zizanioides* were between 72%–94%, 78%–91% and 70%–83%, respectively (Fig. 1b). In accordance to our report previously *V. zizanioides* has been reported as tolerant and high biomass producing plant under saline cultivation (Liu et al. 2016), heavy metal contamination (As, Cd, Cr, Cu, Hg, Ni, Pb, Se, and Zn) (Darajeh et al. 2014; Danh et al. 2006, 2009, 2012; Datta et al. 2011; Caporale et al. 2014; Chen et al. 2004; Yang et al. 2016), contaminated ore and mining areas (Roongtanakiat et al. 2008; ANZ 1992) and industrial wastewater treatment (Ghosh et al. 2015; Yeboah et al. 2015).

Antimony and arsenic contents in the digested roots, and shoots in Sb, As and Sb + As contaminated *V. zizanioides*, and in the Hoagland solution are presented in Fig. 2a, b. Metal uptake depends upon the genetic makeup of the plant. It varies, within and among plant species (Mirza et al. 2011; Chai et al. 2016, 2017). In Sb (Sb_{10}, Sb_{20}, Sb_{40} and Sb_{80} mg L^{-1}) and As (As_{10}, As_{20}, As_{40} and As_{80} mg L^{-1}) contaminations, the concentrations of Sb and As in the roots and shoots of *V. zizanioides* were significantly increased ($\rho < 0.05$) (Danh et al. 2012), with the increase in applied Sb and As (Fig. 2a, b). In low, moderate and high Sb + As contaminations, a simultaneous steady significant rise ($\rho < 0.05$) in both the Sb and As concentrations in the roots and shoot of *V. zizanioides* were observed. As a result of the plant accumulation, Sb and As concentrations in the Hoagland solution were significantly decreased ($\rho < 0.05$). The trend of the accumulation of both of the contaminants, i.e., Sb and As in *V. zizanioides*, in Sb and As alone and in Sb + As contaminations was as $Root_{[Sb/As]} > Shoot_{[Sb/As]} > Hol.\ sol._{[Sb/As]}$.

Vetiveria zizanioides accumulated, more Sb and As in the roots than in the aerial parts. After 21 days of application of Sb alone (Sb_{10}, Sb_{20}, Sb_{40} and Sb_{80} mg L^{-1}), highest Sb accumulations in the root, and shoots of *V. zizanioides* were 307 ± 13.0 and 217 ± 23.02 mg kg^{-1} Sb, respectively. As compared to control Sb concentrations in the roots:shoot of *V. zizanioides* at Sb_{10}, Sb_{20}, Sb_{40} and Sb_{80} mg L^{-1}, were 70:2.0, 141:3.4, 194:7.0 and 290:12.40 times greater than control, respectively (Fig. 2a). Arsenic concentrations in the roots:shoot of *V. zizanioides* at As_{10}, As_{20}, As_{40} and As_{80} mg L^{-1}, were 123:2.0, 168:7.0, 231:22.0 and 371:29.0 times greater than control respectively (Fig. 2b).

As compared to control at low, moderate and high Sb + As, Sb concentrations in the roots: shoot were 29:2.0, 43:2.8 and 70:4.2 times greater than control, respectively (Fig. 2a). While as compared to control, As concentration in the roots: shoot 78:3.0, 206:6.3 and 250:15.0 times greater than control, respectively (Fig. 2b). It is revealed that the co-contamination of Sb and As at the same level enhanced the Sb and As accumulations in the roots and shoot of *V. zizanioides*. Hence, Sb and As have positive correlation and in *V. zizanioides* they supplemented the uptake and accumulation of each other.

At the end of the experiment the remaining amounts of the applied Sb, in the Hoagland solution, at Sb_{10}, Sb_{20}, Sb_{40} and Sb_{80} mg L^{-1}, were 60%, 66%, 69% and 70%, respectively (40%, 34%, 30% and 30% of the applied Sb were absorbed by *V. zizanioides*) (Fig. 2a). The remaining As concentrations in the Hoagland solution at As_{10}, As_{20}, As_{40} and As_{80} mg L^{-1}, were 79%, 79%, 72% and 62%, respectively (21%, 21%, 28% and 38% of the applied As were absorbed by *V. zizanioides*) (Fig. 2b), respectively. The remaining Sb + As concentrations in the Hoagland solution at low, moderate and high Sb + As co-contaminations, were 57%+47%, 62%+62% and 54%+53%, respectively (43%+53%, 38%+38% and 46%+47% of the applied As were absorbed by *V. zizanioides*) [Fig. 2a (for Sb) and 2B (for As)], respectively. On comparison, if Sb and As both are applied at equal concentrations then *V. zizanioides* accumulates more As than Sb (1.3 time or 2.5% more As than Sb). But when Sb and As both are applied together at the same rate i.e., Sb + As at low, moderate and high concentrations, *V. zizanioides* accumulates more almost four times (~3.77) more As than Sb. Similar to the our results of *V. zizanioides* Tschan et al. (2009) has reported that under Sb + As contamination, ryegrass, wheat and sunflower plants accumulate three times more As concentration than Sb.

Translocation (TF) and bioaccumulation (BF) factors of metals are assessed to determine the phytoremediation potential of the plant for the specific metal. If the TF and BF for a certain metal in a specific plant are >1.0 and 3.0, respectively, then the plant is considered to suitable for the phytoremediation of that specific metal (Chai et al. 2016; Liu et al. 2014). In our experiment, BF of Sb and As in *V. zizanioides*, under applied Sb, As and Sb + As were higher than the TF. Overall the TF of Sb under applied Sb i.e., Sb_{10}, Sb_{20}, Sb_{40} and Sb_{80} mg L^{-1}, was low i.e., 0.47, whereas BF of Sb was 5.00 (Fig. 2c). The TF of As under applied As i.e., As_{10}, As_{20}, As_{40} and As_{80} mg L^{-1}, was 0.85, whereas BF was 8.00 (Fig. 2d). The TF and BF of

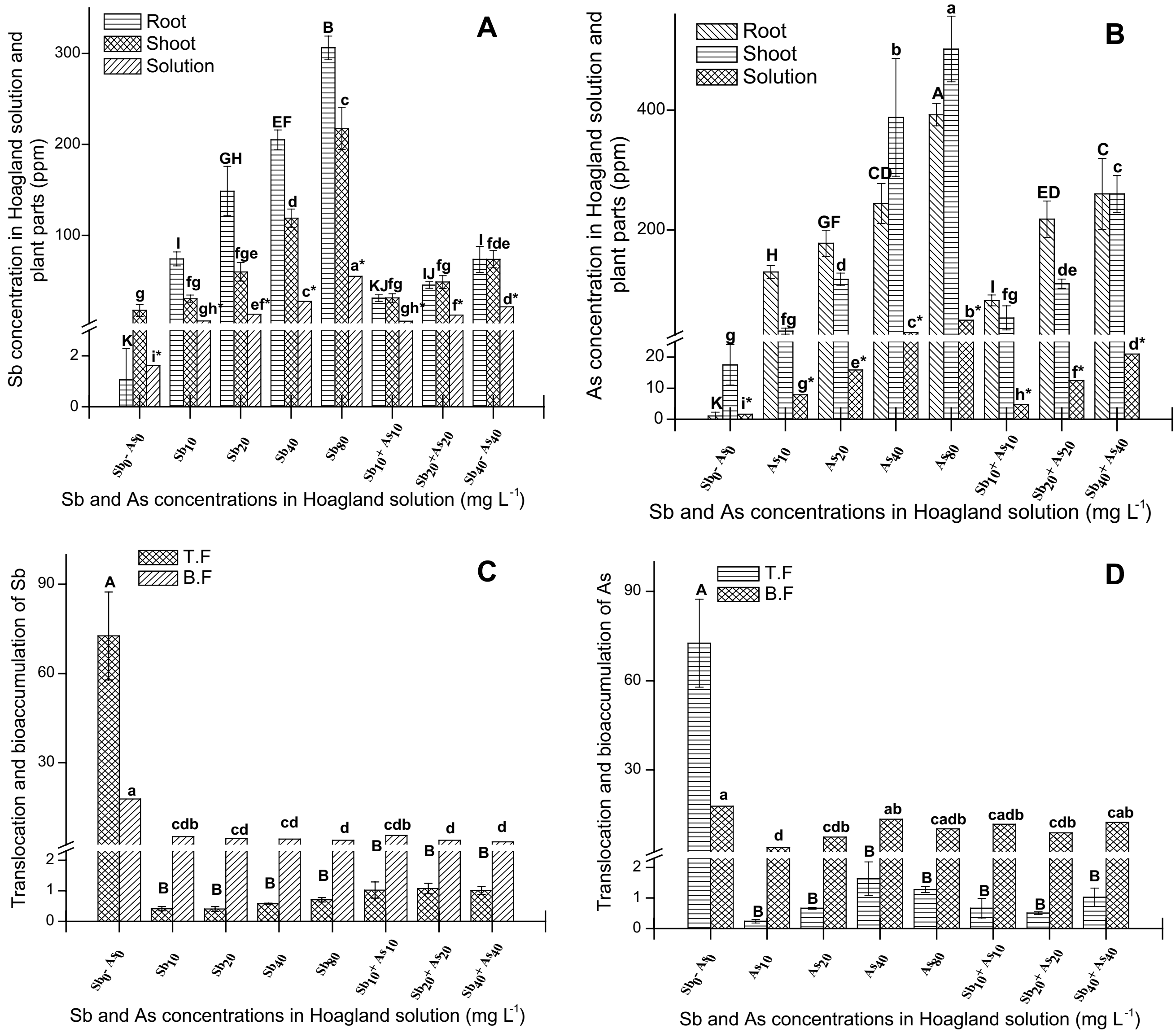

Fig. 2 **a** Effect of Sb and As application, on the Sb concentration in Hoagland solution and plant parts (ppm) of *Vetiveria zizanioides*. **b** Effect of Sb and As application, on the As concentration in Hoagland solution and plant parts (ppm) of *V. zizanioides*. **c** Translocation and bioaccumulation factors of Sb in *V. zizanioides*. **d** Translocation and bioaccumulation factors of As in *V. zizanioides*. Values followed by *different uppercase letters* are significantly different at $\rho < 0.05$, for treatments. Values followed by *different lowercase letters* are significantly different at $\rho < 0.05$, for treatments. *Different letters* indicate significant differences between treatments for each parameter, at significant level of 0.05. Values followed by the *same letters* for each parameter are not significantly different at the 0.05 level (least significant difference). Values in the graph are mean (n = 3), *error bars* are standard deviation (SD)

Sb under low, moderate and high applied Sb + As, were 1.04 and 4.00, respectively (Fig. 2c). While TF and BF of As under applied Sb + As were 0.73 and 11.00 (Fig. 2d). The trends of the TF and BF of both of the contaminants, i.e., Sb and As in *V. zizanioides*, in Sb and As alone and in Sb + As contaminations were as

$$TF - As_{Alone} > TF - As_{Sb+As} > TF - Sb_{Sb+As} > TF - Sb_{Alone}$$

$$BF - As_{Sb+As} > BF - As_{Alone} > BF - Sb_{Alone} > BF - Sb_{Sb+As}$$

The TF of As under As alone i.e., As_{10}, As_{20}, As_{40} and As_{80} mg L^{-1} was two times of the TF of Sb under Sb alone i.e., Sb_{10}, Sb_{20}, Sb_{40} and Sb_{80} mg L^{-1}. The TF of As under low, moderate and high Sb + As, was almost three times of the TF of Sb. In accordance with the results Tisarum et al. (2014) and Pierart et al. (2015) have reported higher bioaccumulation of Sb in *Pteris vittata, Typha latifolia, Scirpus sylvaticus, Phragmites australis*, edible herbs and plants. Okkenhaug et al. (2012) and Feng et al. (2015), have reported higher Sb concentration in the plant parts of rice and four ferns over As, respectively. Higher Sb and As concentrations in the plant parts could be a risk for the life on earth. But the spread of these extracted toxins i.e., Sb and/or As could be avoided by composting and biochar of the harvested shoots. Thus, *V. zizanioides* proved as an efficient candidate for the eco-restoration and remediation of Sb and As contaminated areas.

This study proved the resistance of *V. zizanioides* by inhibited growth, phytotoxic effects, tolerance indices and high Sb and As concentrations in the plant parts. Growth inhibition of *V. zizanioides* was highest under Sb + As co-contamination and was least under As alone. Phytotoxic effects of Sb and As on *V. zizanioides* were highest under Sb + As and were least under of As alone. The concentrations of Sb and As increased with the increase in applied Sb, As alone and Sb + As co-contaminations. In *V. zizanioides* the highest amounts of Sb and or As were found in the roots followed by shoot. When Sb and As alone were applied at equal concentrations then *V. zizanioides* accumulated 1.3 times more As than Sb. But when Sb and As were applied as co-contamination, *V. zizanioides* accumulated almost four times more As than Sb. Translocation (TF) of As in *V. zizanioides* was higher than the TF of Sb both under alone and co-contamination. This study proved *V. zizanioides* as a potential candidate for the eco-restoration of Sb and As contaminated areas. In order to understand the tolerance mechanism of *V. zizanioides* for Sb and As the internal physiological changes of *V. zizanioides* is need to be studied.

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