

## SEED PRIMING WITH SELENIUM TO AFFECT SEED GERMINATION, SEEDLING GROWTH, AND ELECTROLYTE LEAKAGE IN RICE UNDER VANADIUM AND CADMIUM STRESS

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Electrolyte leakage, germination, heavy metals, rice; selenium, vigor indices

### ABSTRACT

**Background** Contamination with heavy metals might cause a severe decline in plant growth and agricultural productivity. Among the plant growth stages, appropriate seed germination is most critical to get optimum yield. Heavy metals at higher concentrations may affect the germination, and thus reduce the plant growth and yield in most of the plant species.

**Methodology** The present study was conducted to evaluate the effect of selenium (Se) against various levels of vanadium (V) and cadmium (Cd) at germination stage. The experiment was planned in controlled environment. Rice seeds were exposed to different levels of V (0, 300, 600 and 1200  $\mu\text{M}$ ) and Cd (0, 100, 250 and 500  $\mu\text{M}$ ), with and without Se priming at 10  $\mu\text{M}$ .

**Results** It was found that V and Cd stress significantly reduced the germination percentage, shoot and root length, shoot length vigor index (SLVI), electrolyte leakage (EL), tolerance index (TI), germination index (GI), protrusion percentage (PP) and mean daily germination (MDG) while, the results about 50% germination of seeds ( $T_{50}$ ) were not highly significant. Moreover, V at 1200  $\mu\text{M}$  and Cd at 500  $\mu\text{M}$  caused more toxicity than lower levels (300 and 600  $\mu\text{M}$  V) and (100 and 250  $\mu\text{M}$  Cd). However, application of Se mitigated the toxic effects of V and Cd, and improved the growth parameters of rice under V and Cd stress.

**Conclusion** Toxicity of V and Cd to rice growth increased with increasing the level of metal application. Selenium application could alleviate the toxic effects of V and Cd. However, the efficiency of Se against V and Cd toxicity was more pronounced at 300, and 600  $\mu\text{M}$  V and 100 and 250  $\mu\text{M}$  Cd as compared to higher levels (1200  $\mu\text{M}$  V and 500  $\mu\text{M}$  Cd).

**Abbreviations:** Electrical conductivity = EC, Electrolyte leakage = EL, Germination percentage = GP, Germination index = GI, Mean daily germination = MDG, Protrusion percentage = PP, Shoot length vigor index = SLVI, Shoot weight vigor index = SWVI, Tolerance index = TI, Time taken for 50% seed germination =  $T_{50}$ , Vigor index = VI

### INTRODUCTION

Among the major environmental pollutants, vanadium (V) and cadmium (Cd) are the most

phytotoxic heavy metals. Both metals are released into air, water and soil through agricultural, industrial or urban activities. Many studies have indicated the high values of V and Cd in agricultural fields,

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particularly due to the long-term use of phosphate fertilizers, sewage sludge, and waste water irrigation (Ringelband and Hehl 2000; He et al. 2009; Uraguchi and Fujiwara 2012, Ashraf et al. 2017). These non-essential elements are highly mobile in the soil-plant system which allows their easy entry into plants, can impair several vital processes, and resulting in poor growth and low economic yield of crops. All these ultimately cause toxic effects on human health through the food chain contamination (Ekmekci et al. 2008; Shamsi et al. 2008). Any element which has density  $>5 \text{ g cm}^{-3}$  is considered as heavy metals. The heavy metals can be divided into three categories on the basis of their role in plants; 1) essential metals like zinc (Zn), copper (Cu), iron (Fe), manganese (Mn) and molybdenum (Mo) etc., 2) beneficial metals like cobalt (Co), 3) harmful metals like V, Cd, and lead (Pb) etc. (Marschner 2012). The higher concentrations of heavy metals can affect the enzyme activities and plant metabolism, causing toxic effects on plants (Shukla et al. 2007).

In the list of heavy metals, V is particularly toxic to different plant species (Panichev et al. 2006; Rosso et al. 2005). Vanadium is considered one of the most important elements of the 21<sup>st</sup> century due to its high consumption in industries and scientific development. Vanadium is extensively distributed in the environment by different ways like leaching, combustion, fertilization, and waste material from industries, and resultantly. V contaminates the soil, water and atmosphere (Ringelband and Hehl 2000).

Cadmium is a highly toxic element that is widespread in the environment from several contamination sources. Consequently, its bioaccumulation through the food chain represents a risk for animal and human health (Stohs et al. 2000). The toxicity of Cd to physiological functions in plants have been widely investigated, and drastic diminutions of biomass production, physiological activities and nutritional quality have been observed in crops grown on soils contaminated with this non-essential element (Benavides et al. 2005; Chaoui and El Ferjani 2005).

The previous reports revealed that heavy metals badly affected the imbibition and germination of seeds and also penetrated the seed coat and involved in the inhibition of physiological processes associated with germination and embryo development processes (Seregin and Kozhevnikova 2005). Actually, metals affect the seed germination by two ways; 1) due to toxicity and 2) effect on water uptake (Kranner and Colville 2011). Especially, seed germination and subsequent embryo development are important stages of the plant life which are highly sensitive to surrounding medium fluctuations, because the

germinating seed is the first interface of material exchange between plant development and environment. Delay in germination has often been observed after heavy metals exposure (Smiri et al. 2009). This can be associated with disorders in the event chain of germination metabolism, which is a highly complex multistage process, itself coordinated by physiological, biochemical and molecular programs.

In the present study, ecotoxicological effects of V and Cd were investigated for seed germination assay of rice (*Oryza sativa* L.). Currently, no data are available about the effect of Se on seed germination in rice under V and Cd stress. The present study was carried out to assess the hypothesis that Se may alleviate the V and Cd toxicities via germination, shoots lengths, and vigor indices.

## MATERIALS AND METHODS

### *Plant material and stress conditions*

The healthy rice seeds were surface sterilized with 2% NaClO solution for 15 minutes, and then washed many times with distilled water. The seeds were divided into two groups; seeds in group-I were put in 10  $\mu\text{M}$  solutions of Se for 24 hours, and seeds in group-II were put in distilled water (without Se) for 24 hours. After 24 hours, 50 seeds were put in a petri dish of 20 cm diameter. The bottom of each petri dish was covered with two layers of filter paper. After putting the seeds in each petri dish, 5 ml of distilled water (control: soaked separately in water, and in Se), V solution at 300, 600 and 1200  $\mu\text{M}$  in the form of ammonium metavanadate ( $\text{NH}_4\text{VO}_3$ ) and Cd solution at 100, 250 and 500  $\mu\text{M}$  in the form of cadmium nitrate [ $\text{Cd}(\text{NO}_3)_2$ ] were added to the respective petri dish according to treatment plan. The experiment was designed according to completely randomized design (CRD) with three replicates for the period of 18 days. The growth room was climate-controlled with a temperature range 22-25°C and relative humidity 70%. Vanadium and Cd solutions and distilled water were replaced every day to maintain the concentration of the treatment solutions constant as well as to supply the optimum moisture for the germination of the seeds.

### *Variants of the experiment*

The measurement of the protrusion (visible emergence of primary root from seed coat) and germination (primary root size  $\geq 2$  mm length) of the seeds were taken daily basis for first 7 days. Some germinated seeds were discarded from each petri dish, however, only the seeds those were under the process of germination and some germinated leave to remain in

petri dishes. No more germination was considered after the 11<sup>th</sup> day of the experiment, ten germinated seeds were used from each petri dish for further analysis such as shoots lengths and vigor indices.

**Measurement of parameters**

For mean and variation of germination time of the seeds, the percentage of the protrusion (PP) and percentage of final germination (GP) were calculated according to the Akinci and Akinci (2010):

$$PP = \sum (P/T) * 100$$

$$GP = \sum (G/T) * 100$$

Where:

P = total number of protruding seeds

G = total number of germinated seeds

T = total number of seeds per petri dish

The time to find 50% germination (T<sub>50</sub>) was determined according to the Coolbear et al. (1984) modified by Farooq et al. (2005):

$$T_{50} = t_i + \frac{\left(\frac{N}{2} - n_i\right) (t_j - t_i)}{n_j - n_i}$$

Where N is the final number of germination and n<sub>i</sub>, n<sub>j</sub> cumulative number of seeds germinated by adjacent counts at times t<sub>j</sub> and t<sub>j</sub> when n<sub>i</sub> < N/2 < n<sub>j</sub>.

Germination index (GI) was determined according to the Association of Official Seed Analysts (1983):

$$GI = \frac{\text{No. of germinated seeds}}{\text{Days of first count}} + \dots + \frac{\text{No. of germinated seeds}}{\text{Days of final count}}$$

Tolerance indices (TI) were calculated with the formula as described by Iqbal and Rahmati (1992):

Tolerance index (TI) = Mean shoot length in metal solution/mean shoot length in control x 100

After measuring the shoots and roots lengths and fresh biomass, seedling length vigor index (SLVI) and mean daily germination (MDG) were calculated by the formulas described by Abbasian and Moemeni (2013):

$$SLVI = \text{Mean shoot length} \times FGP$$

$$MDG = \frac{FGP}{d}$$

Where FGP: final germination percent

d: test period

**Electrical conductivity measurements**

The germination media were collected and analyzed for electrical conductivity on daily bases. Special care was taken to remove most of the medium from the filter paper by folding the paper and applying pressure. Electrical conductivity measurements were carried out using a conductivity meter. The electrical conductivity of germination media was expressed on the basis of seed number (μohm cm<sup>-1</sup> seed<sup>-1</sup>) in accordance with Powell and Raymond (1981).

**Statistical analysis**

All the collected data were subjected to analyze using SAS software. Analysis of variance (ANOVA) and the treatments means comparison was made by Duncan's Multiple Range Test (DMRT) to determine the level of significance at *p* ≤ 0.05.

**RESULTS**

The results of the present study indicated that higher concentrations of both metals, V and Cd badly affected the germination of seeds and growth parameters of rice seedlings. Compared to the control, the PP was gradually reduced with the increase of V and Cd concentrations (Figure 1). The maximum reduction was occurred about 52% and 60% against 500 and 1200 μM of Cd and V, respectively when treated without Se-priming. However, seed priming with Se significantly increased the PP. The Se-priming of rice seeds effectively increased the PP against all levels of both metals. However, the effect of Se-priming against higher concentrations of Cd (500 μM) and V (1200 μM) was the lowest. Overall, the rice seed-priming with Se alleviated the Cd and V toxicity and improved the PP (Figure 1).

The GP for controls and treated genotypes are presented in Figure 2. The lowest GP was recorded at higher levels of Cd and V. The GP was reduced by 48% and 58% when the rice seeds were exposed to 500 μM of Cd and 1200 μM of V, respectively without Se-priming. While, seed-priming with Se effectively increased the GP of rice, however, the alleviation effect of Se-priming at higher levels of Cd and V was recorded lowest. Generally, Se-priming enhanced the GP against both metals stresses.

The effect of Cd and V stress on GI of rice seed grown at different Cd and V levels with and without Se-priming was variable and concentration/or Se-priming dependent (Figure 3). The GI was gradually declined with the increasing concentration of both metals. The highest decline of GI was recorded at

500  $\mu\text{M}$  of Cd and 1200  $\mu\text{M}$  of V which was 10.8 and 8.5, respectively without Se-priming. On the other hand, seed-priming with Se increased the GI against all the levels of Cd and V.

The MDG of rice seeds were significantly ( $p \leq 0.05$ ) decreased when treated with Cd and V concentrations without Se-priming as compared to control, and the highest decrease was confirmed at higher concentration of Cd (500  $\mu\text{M}$ ) and V (1200  $\mu\text{M}$ ) with respect to control (Figure 4). While, seed-priming with Se significantly improved the MDG against all levels of Cd and V but the lowest effect of Se-priming was observed against higher doses of both metals.

The results about shoot length, plant biomass, SLVI and SWVI are presented in Figure 5, 6, 7 and 8. The results showed that shoot length was significantly ( $p \leq 0.05$ ) higher in control as compared to plants exposed to Cd and V without Se-priming and increasing concentration of both metals drastically reduced the length and biomass. The minimum length and biomass of rice seedlings were recorded at 500  $\mu\text{M}$  Cd and 1200  $\mu\text{M}$  of V in the absence of Se-priming which was 3.1 cm and 1.9 cm, 2.1, and 1.1 g respectively (Figure 5 and 7). The SLVI and SWVI were also significantly ( $p \leq 0.05$ ) reduced in Cd and V treated seedlings than control as well as treated with both metals plus Se-priming. The reduction in SLVI was observed about 7.4 and 14.3 folds while, SWVI was reduced about 7.2 and 16.4 folds as compared to control when seedlings were exposed to 500  $\mu\text{M}$  of Cd and 1200  $\mu\text{M}$  of V (Figure 6 and 8). However, the shoot length, SLVI, biomass and SWVI of the seedlings grown after seed-priming with Se showed tolerance to the toxicities of both metals.

Results for the  $T_{50}$  grown at different concentrations of Cd and V are presented in Figure 9. Application of Cd and V to rice seeds not treated with Se significantly ( $p \leq 0.05$ ) affected the  $T_{50}$ . Both Cd and V application at 100 and 250  $\mu\text{M}$  and 300 and 600  $\mu\text{M}$  considerably increased the  $T_{50}$  of rice seeds. The rice seeds only treated with Cd and V metals took long time to achieve 50% germination. It was found that 6.1 days was spent to complete  $T_{50}$  when seeds were treated with Cd at 500  $\mu\text{M}$  and 50% of seed germination was completed when treated with 1200  $\mu\text{M}$  of V (Figure 9). On the other hand, the  $T_{50}$  was completed early when seeds were exposed to lower concentrations of Cd and V. However, seeds treated with Se effectively reduced the time period to complete the  $T_{50}$ , especially at lower levels of Cd and V. Even, Se-priming alleviated the V toxicity and completed the 50% of seed germination that was not achieved without Se-priming (Figure 9).

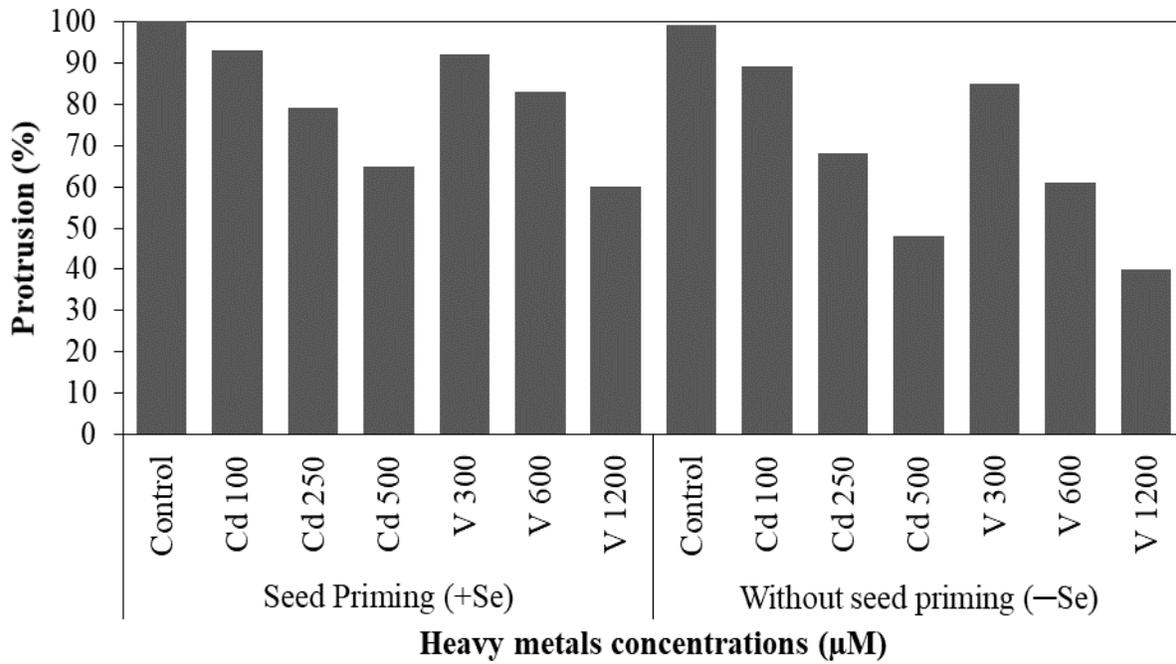
The results about electrolytes leakage or damage in seed coat are presented in Figure 10. Cadmium and V solution were taken four times to check the electrolyte leakage via electrical conductivity. The maximum electrolyte leakage was observed at 4<sup>th</sup> sampling that showed the interaction of the metals solution for a long time could badly damage the seed coat. The maximum electrolyte leakage was observed when seeds were exposed to higher concentrations of Cd and V without Se-priming. While, Se-priming of seeds helped to stabilize the seed coat and reduced the electrolyte leakage, even after 12 days of germination (Figure 10).

## DISCUSSION

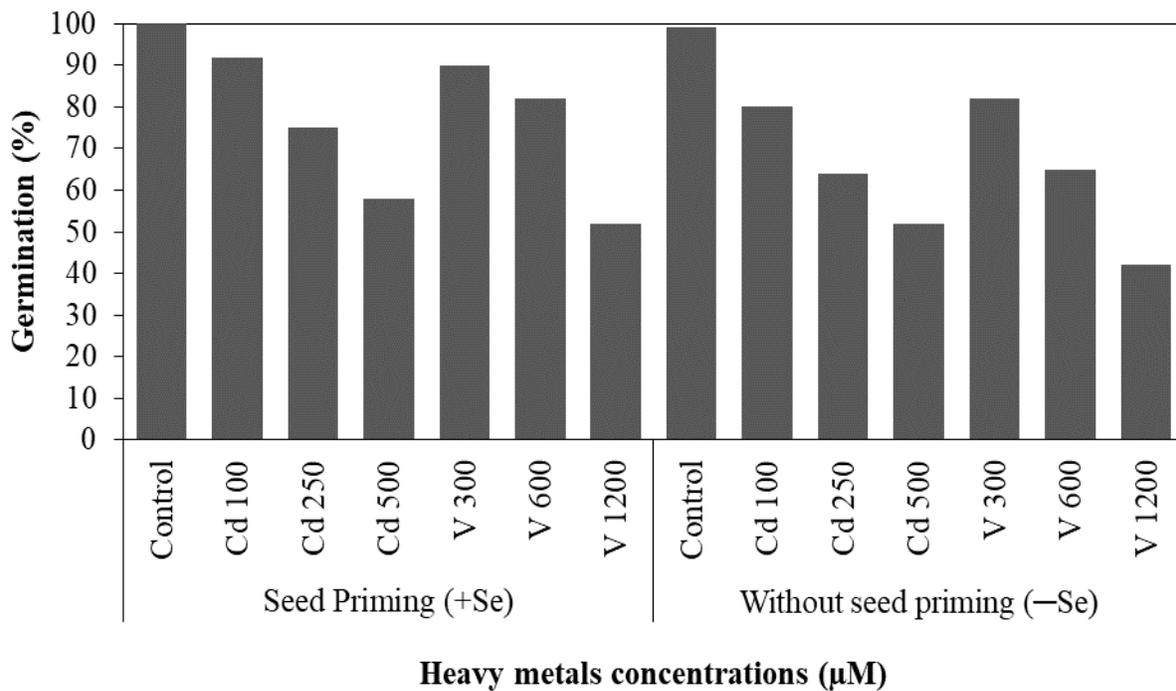
The application of Cd and V caused significant effects on the PP of rice seeds. Heavy metals inhibited the cell division in seeds which could affect the PP. This reduction of PP under metal stress might be due to disruption of seed coat by metal toxicity, due to which metals entered the seeds and affected the seed metabolism (Khaliq et al. 2015). Similar results were reported by Seregin and Kozhevnikova (2005) and Kranner and Colville (2011) regarding the PP of seeds under metal stress.

The growth parameters like GP, GI (Mhatre and Chaphekar 1982) and shoot length were used as an indicator against metal toxicity in plants (Uveges et al. 2002). The differences in GP, GI and shoot length clearly showed the toxic effects of Cd and V on rice seed germination. The GP, GI and shoot length were not inhibited at lower concentrations of Cd and V, indicating that lower levels of Cd and V were not more toxic at the germination stage. However, higher concentration detrimentally reduced these parameters indicating that higher levels of both metals were more toxic for seed germination. The MDG was also strongly affected by Cd and V application, however, at lower concentrations of both metals, the reduction in MDG was not significant. The results of the present study strongly agreed to the findings of other researchers (Hoshmandfar and Moraghebi 2011).

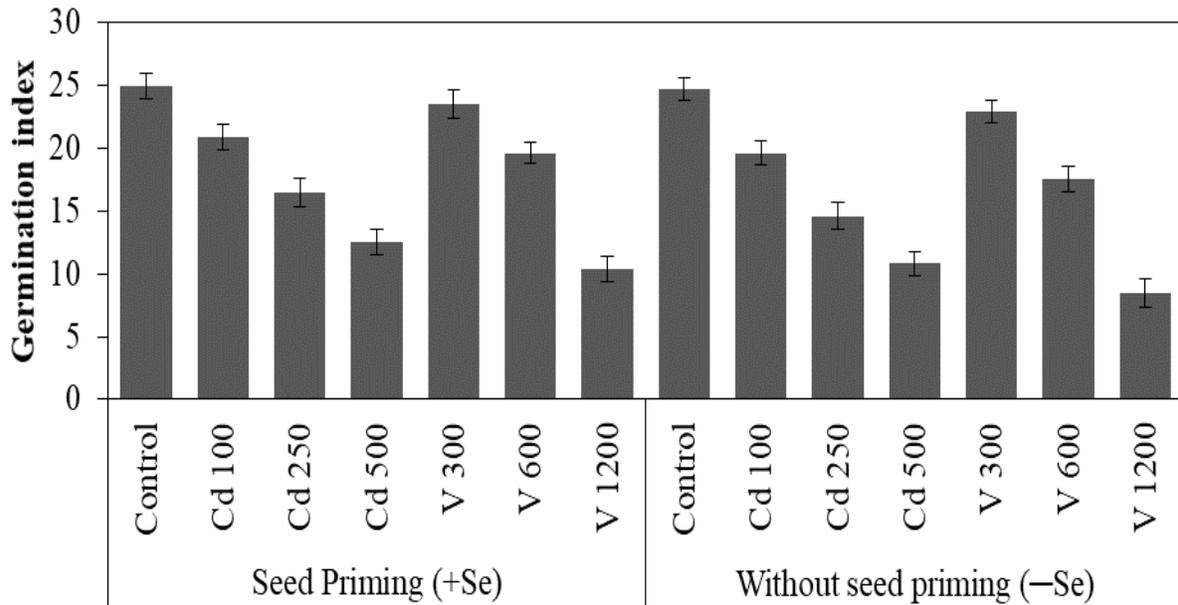
Rahman and Mahmud (2010) reported that higher concentrations of Ni and Co also caused a reduction in GP in plants. This reduction in GP might be due to depression of oxygen uptake and disturbance in the transportation of stored food materials in seed, and damage the selective properties of cell membrane (De Andrade and da Silveira 2008). These results showed conformity with the results of other researchers (Rout et al. 2000; Akinci and Akinci 2010). Like plant species, plant organs also differed for metals accumulation and adaptability



**Figure 1** Effects of cadmium (Cd) and vanadium (V) with and without selenium priming on protrusion percentage (PP) of rice seeds

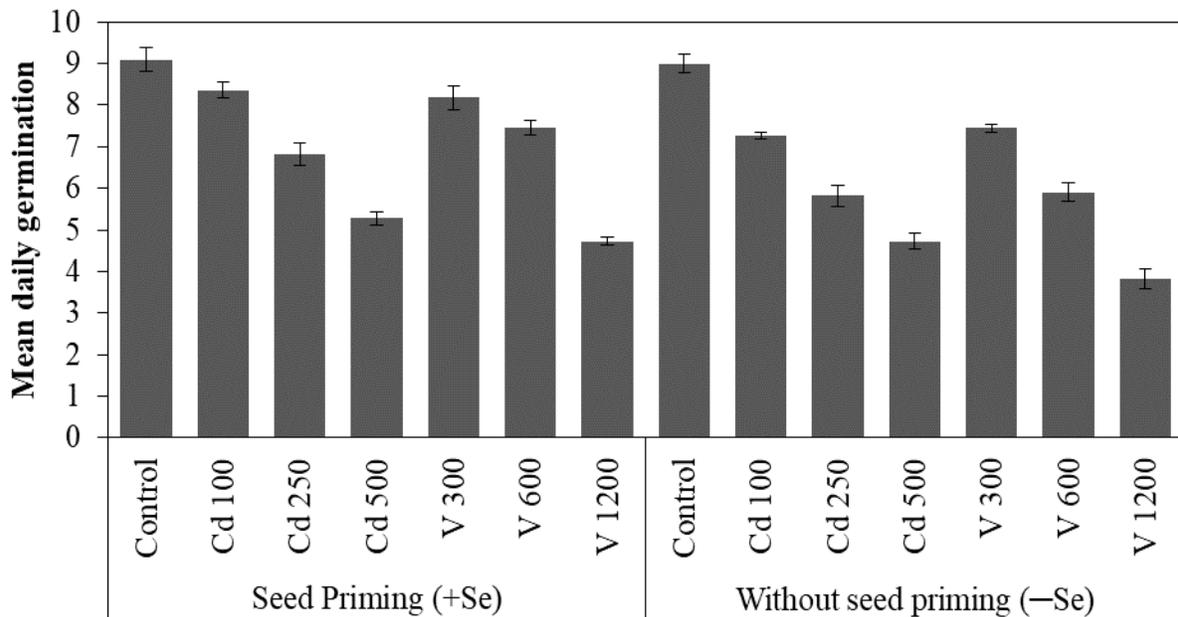


**Figure 2** Effects of cadmium (Cd) and vanadium (V) with and without selenium priming on germination percentage (GP) of rice seeds



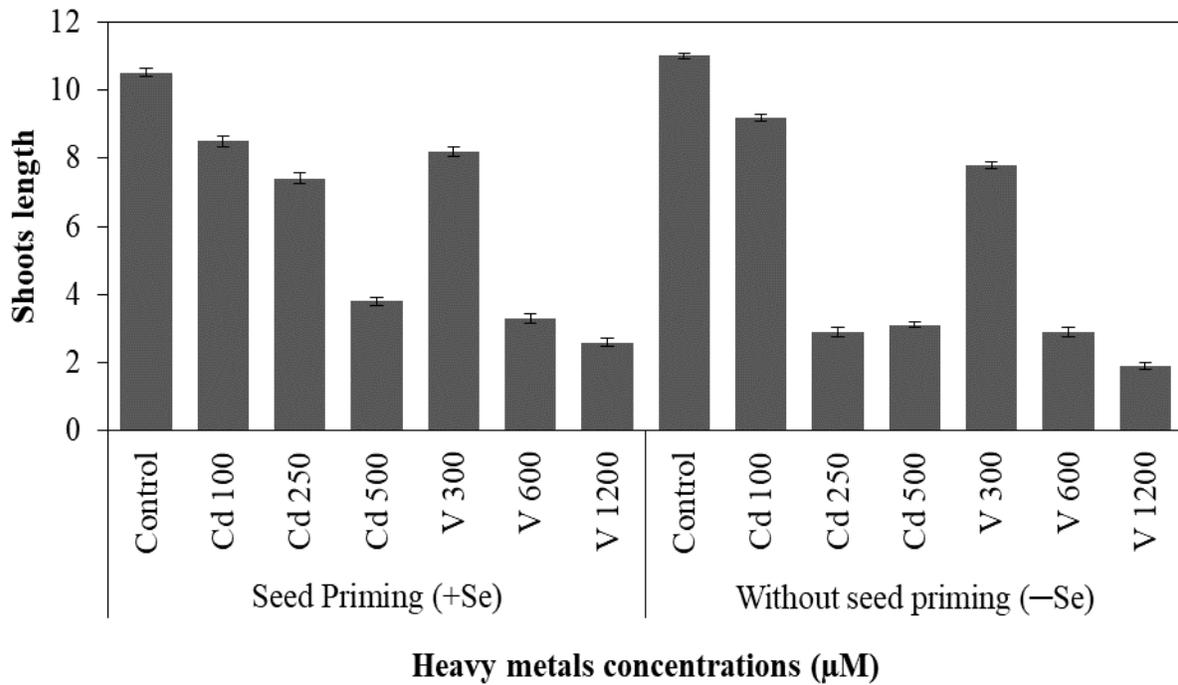
**Heavy metals concentrations ( $\mu\text{M}$ )**

**Figure 3** Effects of cadmium (Cd) and vanadium (V) with and without selenium priming on germination index (GI) of rice seeds. Values represent means  $\pm$  S.D. (n = 3)

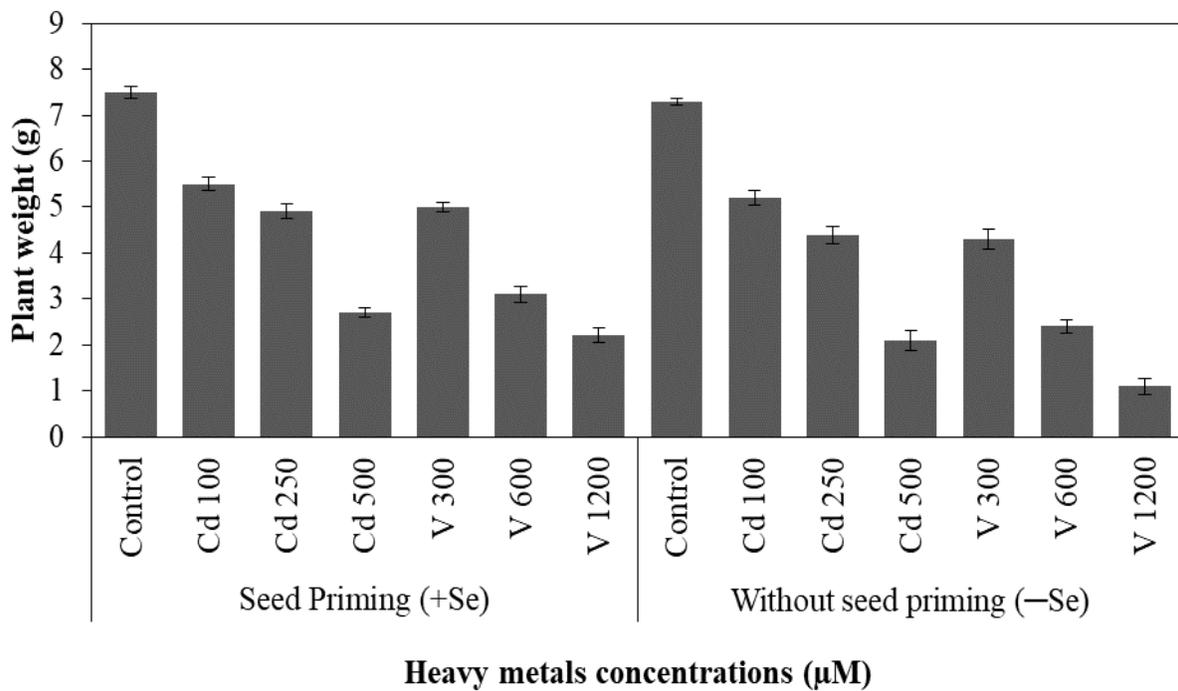


**Heavy metals concentrations ( $\mu\text{M}$ )**

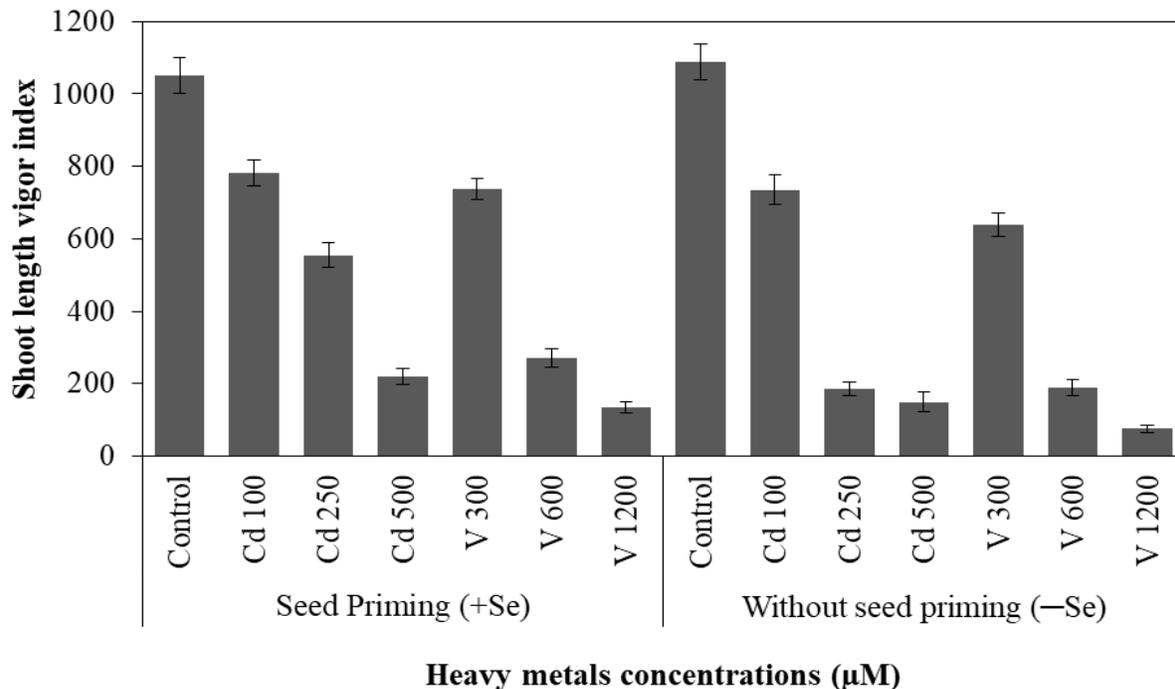
**Figure 4** Effects of cadmium (Cd) and vanadium (V) with and without selenium priming on mean daily germination (MDG) of rice seeds. Values represent means  $\pm$  SD (n = 3)



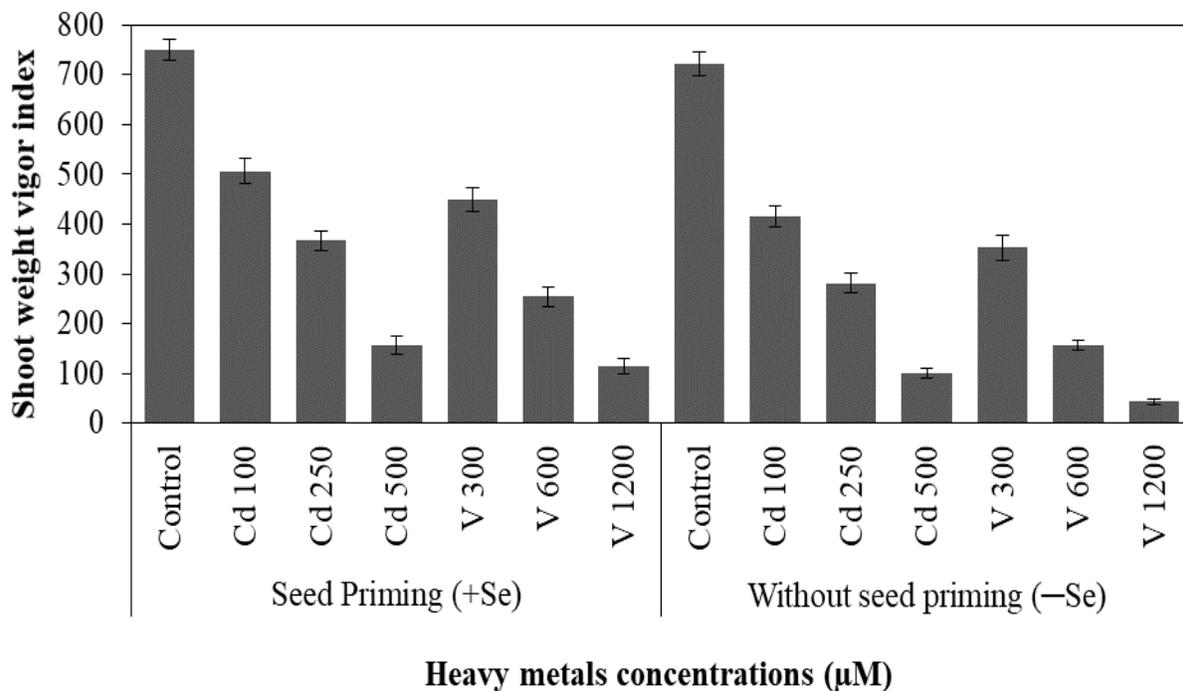
**Figure 5** Effects of cadmium (Cd) and vanadium (V) with and without selenium priming on shoot length of rice seedlings. Values represent means  $\pm$  SD (n = 3)



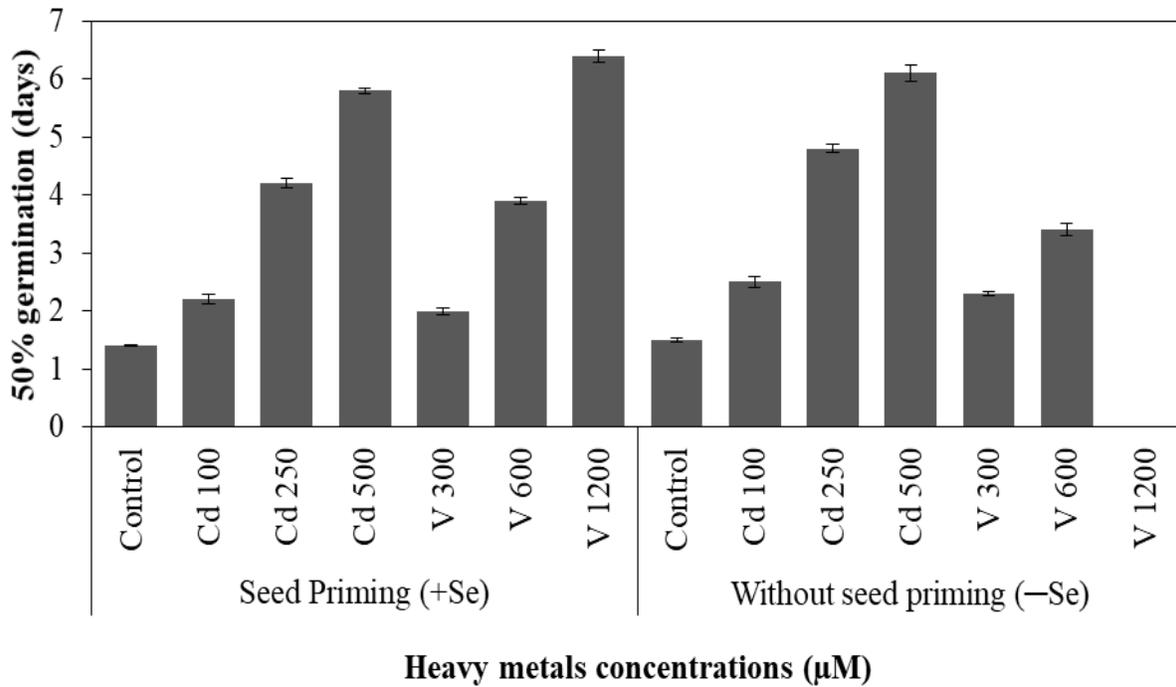
**Figure 6** Effects of cadmium (Cd) and vanadium (V) with and without selenium priming on plant weight of rice seedlings. Values represent means  $\pm$  SD (n = 3)



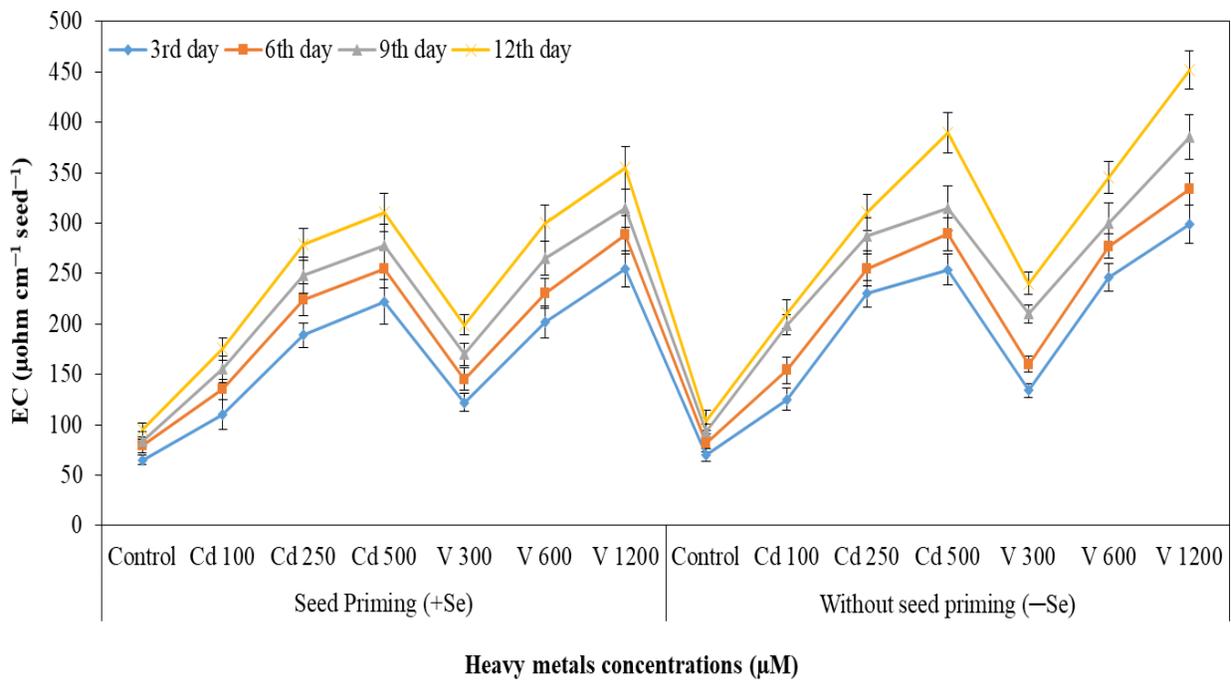
**Figure 7** Effects of cadmium (Cd) and vanadium (V) with and without selenium priming on shoots length vigor index (SLWI) of rice seedlings. Values represent means ± SD (n = 3)



**Figure 8** Effects of cadmium (Cd) and vanadium (V) with and without selenium priming on shoot weight vigor index (SWVI) of rice seedlings. Values represent means ± SD (n = 3)



**Figure 9** Effects of cadmium (Cd) and vanadium (V) with and without selenium priming on time taken to complete 50% rice seeds germination. Values represent means  $\pm$  SD (n = 3)



**Figure 10** Electrical conductivity (EC) of germination media of rice seeds after imbibitions with water, cadmium and vanadium with and without selenium priming. Values represent means  $\pm$  SD (n = 3)

(Ramos et al. 2002). Therefore, there was difference in rice seed germination. These results are in line with the findings of the earlier researchers (Raziuddin et al. 2011; Khaliq et al. 2015; Arezoo et al. 2016).

Higher concentration of cadmium (Cd) and vanadium (V) badly affected the seed germination as well as  $T_{50}$ , and  $T_{50}$  showed a positive relation with increasing concentration of vanadium. Moreover, 500 of Cd and 1200 of V maximally delayed the  $T_{50}$  of rice seeds treated without Se-priming among all the treatment. The metal toxicity causes bad effects on seed coat and metabolism, and affect the permeability of cell membrane inside the seeds (Shafiq et al. 2008), this factor might be the main reason to increase the time period for  $T_{50}$ . Selenium treatment improved the  $T_{50}$  and these results were same in line with Khaliq et al. (2015).

The plant growth (e.g. height and biomass) is the best indices against metal stress. The findings of the present study indicated that cadmium (Cd) and vanadium (V) stress extensively affected on said traits. Our findings indicated that application of Cd and V affected the GP; the seeds that attained the germination state were then subjected to continuous exposure of added metals, showed the clearly stunting of heights and biomass.

The shoot elongation of rice seedlings reduced significantly ( $p \leq 0.05$ ) in all treatments which were not undergoing Se-priming with respect to seeds treated with Se. The reduction in shoot length at higher concentrations of Cd and V could be attributed due to decreasing of meristematic cells and enzyme activities present in cotyledon and endosperms (Shafiq et al. 2008). These results also showed an agreement with the results of Street et al. (2007) and Liu et al. (2008). Effects of different levels of Cd and V on plant weight were also evaluated. The plant weight was notably reduced with increasing of Cd and V levels. The plant weight made a negative relationship with metals applied without Se-priming in growth medium. The previous researchers also reported that application of higher concentration of heavy metals declined the seedlings growth and biomass yield (Ozdener and Kutbay 2009; Solanki and Anju 2011; Wei et al. 2012). These findings strongly indicated a negative relationship between plant weight and metal treatments and are in line with the results of Chongkid et al. (2007) and Xi-yuan et al. (2012). The reduction in shoot growth due to Cd and V was the main factor to decrease the SWVI. Higher concentration of metals damaged the cell division and other main physiological mechanisms which could be the reason for the reduction in SWVI (Clemens, 2001). The obtained findings are in line with Sharikova et al. (2003) and Khaliq et al. (2015).

The exudation of electrolytes from rice seeds can be correlated with the increasing concentration of both metals (Cd and V). It possibly reflected the leakage out of all soluble cell ingredients as indicated by the extent of Cd and V enhanced EC in the germination medium (Figure 10). There may be several factors involved in this damage of seed coat such as membrane damage and loss of essential elements (Powell and Raymond 1981). The exudates of electrolytes from plants are mainly the reflection of impairment of cell membrane and ultimately poor retention of solutes, resulting in leakage from imbibing seeds and this happened mainly because of rupture of cell membranes. This kind of damage in cell membrane could be mediated by metals stresses, which regulated the oxidative injury and this injury impaired the membrane structure because of lipid peroxidation (Chaoui and El Ferjani 2005; Ahsan et al. 2007; Agrawal and Mishra 2009; Posmyk et al. 2009).

## CONCLUSIONS

Cadmium and vanadium had inhibitory effects on rice seed germination and initial seedling growth. It was concluded that addition of metals in the growth medium, particularly in higher concentrations significantly reduced the seed germination, plant weight, shoot length and weight vigor indices, and postponed the time to accomplish the 50% germination. However, Se-priming of rice seeds significantly improved the all observed parameters which showed that Se could enhance the tolerance against metals toxicities. In combined polluted areas, further study is needed to examine the various levels of metals including Cd and V in the environment and different plant parts.

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