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Original Article

Effect of Vitamin B Complex on some Biochemical Parameters of *Aloe vera* L. under Nickel and Cadmium Stress

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Abstract

Nickel and cadmium are heavy metals with some hazards for plant metabolism. The impacts of nickel chloride (0, 400 and 800 µM) in the presence of vitamin B complex (0, 30 and 60 ml from pharmacy commercial stock) and the impacts of cadmium chloride (0, 100, 200, 300, 400, 500 and 600 µl from 100 µM stock) in pot and cadmium nitrate (0, 100, 200, 400, 800, 1600 and 3200 µl from 100 µM stock) in pot on fresh weight, photosynthetic pigments including chlorophyll and carotenoid, total protein and free proline content of *Aloe vera* L. seedlings were investigated. The inhibitory effects of nickel and cadmium on seedling growth resulted in decrease of chlorophyll and total protein contents. Free proline increased, while the fresh weight of the seedling was not affected by the treatments. The results indicate that vitamin B complex alleviated the inhibitory effects of nickel on *A. vera* L. seedlings by increasing chlorophyll, fresh weight and total protein contents.

Key words: *Aloe vera* L., Vitamin B complex, Heavy metals, Medicinal plants, Physiological response.

Introduction

Aloe belongs to Liliaceae, the family of perennial tropical plants of African origin. More than 360 species are known worldwide. One of the important species of *Aloe* which have been used as folk medicine is Curacao Aloe (*Aloe barbadensis* or *Aloe vera* L.). Records of the use of *A. vera* L. as folk medicine date back to antiquity with an early account from around 1500 B.C. The exudates of *A. vera* L. are used for numerous medical and cosmetic applications since ancient times [1]. The *A. vera* L. gel possesses various biological and physiological activities viz. healing ability of skin burns and cutaneous injuries; prophylactic effect against radiation leucopenia; anti-ulcer; inhibitory action against some bacteria and fungi; inflammation-inhibiting effect [2].

In an analogous manner to general stress theory, mechanisms leading to heavy-metal tolerance can be divided into following groups: avoidance strategies and tolerance strategies. Avoidance mechanisms limit the uptake of heavy metals, thus excluding them from plant tissues. Plants with tolerance mechanisms are capable of accumulating, storing, and immobilizing heavy metals by binding them to amino acids, proteins, or peptides [3]. Nickel (Ni) and cadmium are heavy metals that could be harmful for plant metabolism.

Nickel chloride is the most common of nickel compounds. The Ni content in soil can be as low as 0.2 ppm or as high as 450 ppm in some clay and loamy soils. The average is around 20 ppm, so it is a compound that occurs in the environment only at very low levels. The most common application of Ni is the use as an ingredient of steal and other metal products [4].

Cadmium (Cd), being a highly toxic metal pollutant of soils, inhibits root and shoot growth and yield production [5]. The heavy metal Cd is considered as one of the most dangerous environmental pollutants which usually originates from industrial and agricultural activities such as mining waste disposal and application of pesticides or fertilizers [6,7]. Cd not only inhibits cell division, but also induces damage to different cellular components such as membrane, proteins and DNA [8].

B vitamins are a group of water-soluble vitamins that play important roles in cell metabolism. The vitamin B-complex refers to all of the known essential water-soluble vitamins except for vitamin C. These include

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thiamine (vitamin B_1), riboflavin (vitamin B_2), niacin (vitamin B_3), pantothenic acid (vitamin B_5), pyridoxine (vitamin B_6), biotin, folic acid and cobalamins (vitamin B_{12}). Vitamin B_1, B_2 and B3 play a vital role in cellular energy production. Vitamin B5 influences normal growth and development. Vitamin B6 helps protein break down. Vitamin B_7 helps break down of protein and carbohydrates. Vitamin B_9 assists the cells to maintain DNA. Vitamin B_{12} plays a role in the growth and development.

Natural vitamin B_6 consists of six interconvertible compounds, pyridoxine, pyridoxal, pyridoxamine and their phosphorylated derivatives, pyridoxine 5'-phosphate, pyridoxal 5'-phosphate and pyridoxamine 5'-phosphate [9, 10]. Most bacteria, fungi and plants possess vitamin B_6 biosynthesis pathways, but mammals must acquire the vitamin in their diet. In plants, the *de novo* pathway of vitamin B_6 biosynthesis relies on two proteins, PDX1 and PDX2, which function as a glutamine amidotransferase and produce pyridoxal-phosphate from intermediates of glycolysis and the pentose phosphate pathway. PDX1 and PDX2 work together, with the latter protein as the glutaminase and the former as the synthase domain. Vitamin B_6 plays essential roles as a cofactor in a wide range of biochemical reactions, predominantly in amino acid metabolism. Recently, besides their classical role as coenzymes, a new function has emerged for the various vitamin B_6 compounds in cellular antioxidant defense [11,12].

A link between vitamin B_6 and oxidative stress was originally established in the phytopathogenic fungus *Cercospora nicotianae*. Mutant strains were identified that were particularly vulnerable to their own toxin cercosporin, a photosensitizer that produces singlet oxygen ($^1O_2$) in the light. Unexpectedly, cloning of the mutant genes in *C. nicotianae* revealed that the mutated fungi were affected in a gene of the vitamin B6 biosynthesis pathway. Subsequently, it was shown *in vitro* that vitamin B_6 is able to quench $^1O_2$ with a high efficiency. Additional analyses revealed that vitamin B_6 is also able to quench superoxide. The antioxidant capacities of vitamin B_6 were confirmed in yeast or animal cell cultures supplied with exogenous vitamin B_6 compounds and exposed to different oxidative treatments. Similarly, exogenously applied vitamin B_6 was found to protect plant protoplasts against $^1O_2$-induced cell death. These *in vitro* results indicate that vitamin B_6 is a potential antioxidant and raise the question as to whether plants employ vitamin B_6 to protect themselves against reactive oxygen species (ROS), particularly $^1O_2$ [12].

In this study, effect of vitamin B complex on some biochemical parameters of *Aloe vera* as a medicinal plant, under nickel chloride and cadmium chloride stresses has been investigated.

Materials and Methods

Plant materials and treatments

Seedlings of *A. vera* L. were planted in 0.5 kg pots containing a mixture of perlite and peat moss (3/2, w/w). Each pot contained one seedling. Seven different treatments for cadmium chloride (0, 100, 200, 300, 400, 500 and 600 µl from 100 µM stock) in a pot and cadmium nitrate (0, 100, 200, 400, 800, 1600 and 3200 µl from 100µM stock) in pot were used. The 20 ml volume of these solutions was applied to *A.vera* pots. In addition, in a separate examination, nine different treatments of nickel chloride (400 and 800 µM) and vitamin B complex (30 and 60 ml of pharmacy commercial stock) were used. The applications of vitamin B complex were sprayed on the leaf and or on the soil. The seedlings were allowed to grow for 6 months in a greenhouse at average 27 and 18 °C in day and night), the light and dark photoperiod were 16 and 8 h, respectively. The pots were irrigated twice a week. Fully developed leaves were used for biochemical analysis. Fresh weight was used for analysis of plant growth as g/pot.

Photosynthetic pigments, free proline, and total protein measurement

Leaf chlorophyll was extracted with acetone and measured spectrophotometrically using Lichtenthaler's equation [13]. Leaf proline and total protein were analyzed using 0.1 g of leaf samples according to Bates [14] and Bradford [15] methods, respectively.

Statistical analysis

The experimental designs were randomized complete block with three replicates. The collected data were imported to the Microsoft Excel program for calculations and graphical representation. SPSS software (version 17.0) was used for analysis of variance. The means were compared using Duncan's multiple range tests at $P < 0.05$.

Results and Discussion

Cadmium nitrate, cadmium chloride and nickel chloride (Figs 1, 2 and 3) even at the highest level, could not reduce the *A. vera* L. seedlings fresh weight. Mohsenzadeh *et al.* [16] and Liang *et al.* [17] reported that treatment of maize seedlings with the 100 µM Cd reduced both shoots and root fresh weight significantly. In this study the stocks of cadmium were also 100 µM and it shows that maybe *A. vera* L. is tolerant to heavy metals. In plant, Cd is easily taken up by the root tissues and is accumulated in both roots and shoots [18]. At the cellular level, Cd exerts toxic effects on cell metabolism. Preventation of Cd uptake by plant roots alleviates plant tissues from the adverse effects of this toxic metal [18]. Other reports showed that accumulation of Ni seriously affects the yield of plants, significantly decreasing the weight and the number of seeds [19]. The total dry matter of roots and shoots may also decrease when plants are stressed by Ni [20]. The reduction in the number of flowers and fruits also has reported [21].

Chlorophyll pigments, as the main component of the photosynthetic apparatus, play a pivotal role in plant metabolism and energy supply. In our study, high levels of cadmium nitrate (1600 and 3200 µl from 100µM stock in pot) reduced the content of chlorophyll a, significantly. The content of the chlorophyll b was not affected by cadmium nitrate. The carotenoid content increased in response to the highest level of cadmium nitrate (3200 µl from 100µM stock in pot) (Fig 1A). The content of chlorophyll a was reduced in response to 500 and 600 µl/pot cadmium chloride as compared to 200 µl from 100µM stock. Chlorophyll b content reduced in response to the 600 µl from 100µM stock cadmium chloride. Carotenoid content was not influenced by different amounts of cadmium chloride (Fig 1B).

Chlorophyll a and b content increased in response to application of 30 ml vitamin B complex that sprayed on the leaves or 60 ml/pot vitamin B complex or 0 µl/pot nickel chloride and reduced in response to 60 ml vitamin B complex that sprayed on the leaves or 400 and 800 µl from 100µM stock nickel chloride. Carotenoid content increased in response to application of 60 ml/pot vitamin B complex and reduced in response to 400 and 800 µl from 100µM stock nickel chloride with application of 60 ml vitamin B complex that sprayed on the leaves (Fig 1C). Zengin *et al.* [22] have reported that high Cd levels inhibit the leaf chlorophyll content. Inhibition of carotenoid pigments as protectants of photodynamic damage to the chloroplasts apparatus by Cd could also result in less leaf chlorophyll content [23]. The influence of Ni on photosynthesis is pervasive, occurring both in isolated chloroplasts and whole plants [24]. Ni damages the photosynthetic apparatus at almost every level of its organization, including destroying cells of mesophyll and epidermal tissue and decreasing chlorophyll content [25]. Nickel also damages the thylakoid membrane and the chloroplast grana structure, reducing the size of grana and increasing the number of non-appressed lamellae. Carotenoid is the second most abundant pigment in nature and consists of more than 700 members. Carotenoids are plant pigments that function as antioxidants, hormone precursors, colourants and essential components of the photosynthetic apparatus. Carotenoids accumulate in nearly all types of plastids, not just the chloroplast, and are thus found in most plant organs and tissues, albeit at trace levels in some tissues [26]**.**

Free proline content increased in the high levels of cadmium nitrate (1600 and 3200 µl from 100µM stock) as compared to control. Cadmium chloride did not change the free proline content of *A.vera* L. seedling leaves. Free prolin proline content increased in response to 800 µl/pot nickel chloride in the presence of 30 ml vitamin B complex sprayed on leaves, 0 nickel chloride in the presence of 60 ml vitamin B complex sprayed on leaves and 800 µl from 100µM stock nickel chloride in the presence of 30 ml/pot vitamin B complex. Mohsenzadeh *et al.* [16] have reported that there was a 60% increase in the proline content of the Cd-stressed plants. Proline also accumulates in higher plants in response to ROS production and protects plants against the oxidative damage caused by free radicals [27]. Proline is known to activate the Krebs cycle reactions, thus enhancing the plants energy turnover [28].

vitamin B complex sprayed on *A. vera* leaves reduced the total protein content (Fig 4A, B, and C).

Abiotic stress may inhibit the synthesis of some proteins and promote others [29]. Total proteins were significantly decreased by increasing nickel sulphate in two cultivars of *Raphanus sativus* [30]. Singh and Sinha (2005) found decrease in soluble protein content in *Brassica juncea* when grown on various amendments of tannery waste containing heavy metals [31].The decrease in protein content may cause by enhanced protein degradation as a result of increased protease activity under stress conditions [32]. Also, these heavy metals may have induced lipid peroxidation and fragmentation of protein due to the toxic effects of reactive oxygen species leading to a reduction in protein content [33].Such inhibitory effects of high levels of Ni have been reported to be the result of protein synthesis inhibition and changes in carbohydrate metabolism.

Increasing evidences suggest that Ni toxicity in plants is also associated with oxidative stress [34]. Excessive Ni leads to significant increases in the concentration of hydroxyl radicals, superoxide anions, nitric oxide and hydrogen peroxide [35]. Since Ni is not a redox active metal, it cannot directly generate these reactive oxygen species (ROS).

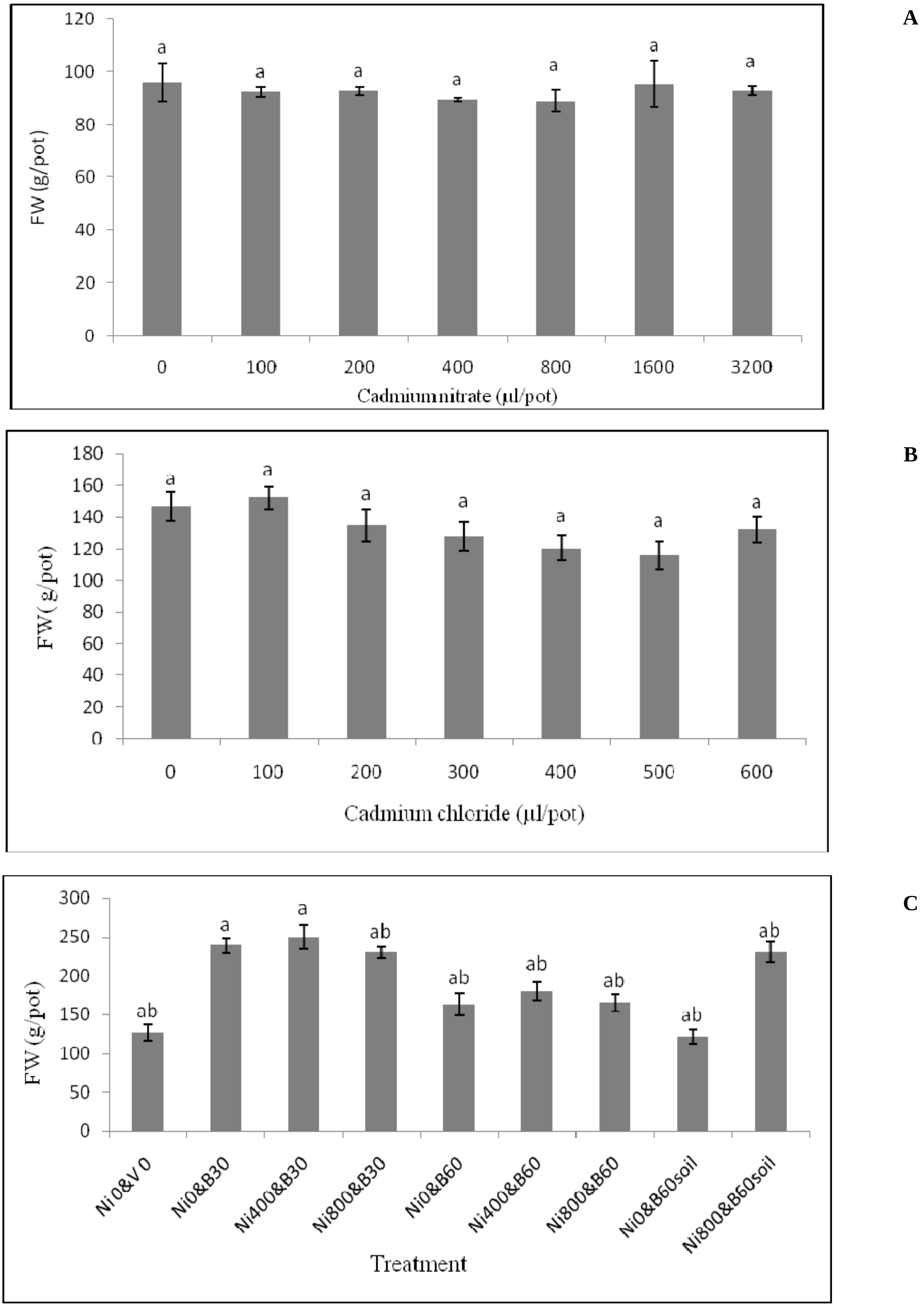

Fig 1 Effect of cadmium nitrate (0, 100, 200, 400, 800, 1600 and 3200 µl from 100µM stock) (A), cadmium chloride (0, 100, 200, 300, 400, 500 and 600 µl from 100µM stock) (B) and nickel chloride (400 and 800 µM) in the presence of 30 and 60 ml from commercial stock of vitamin B complex (C) on *A. vera* L.seedlings fresh weight. Different small letters show significant differences between means at $P \leq 0.05$.

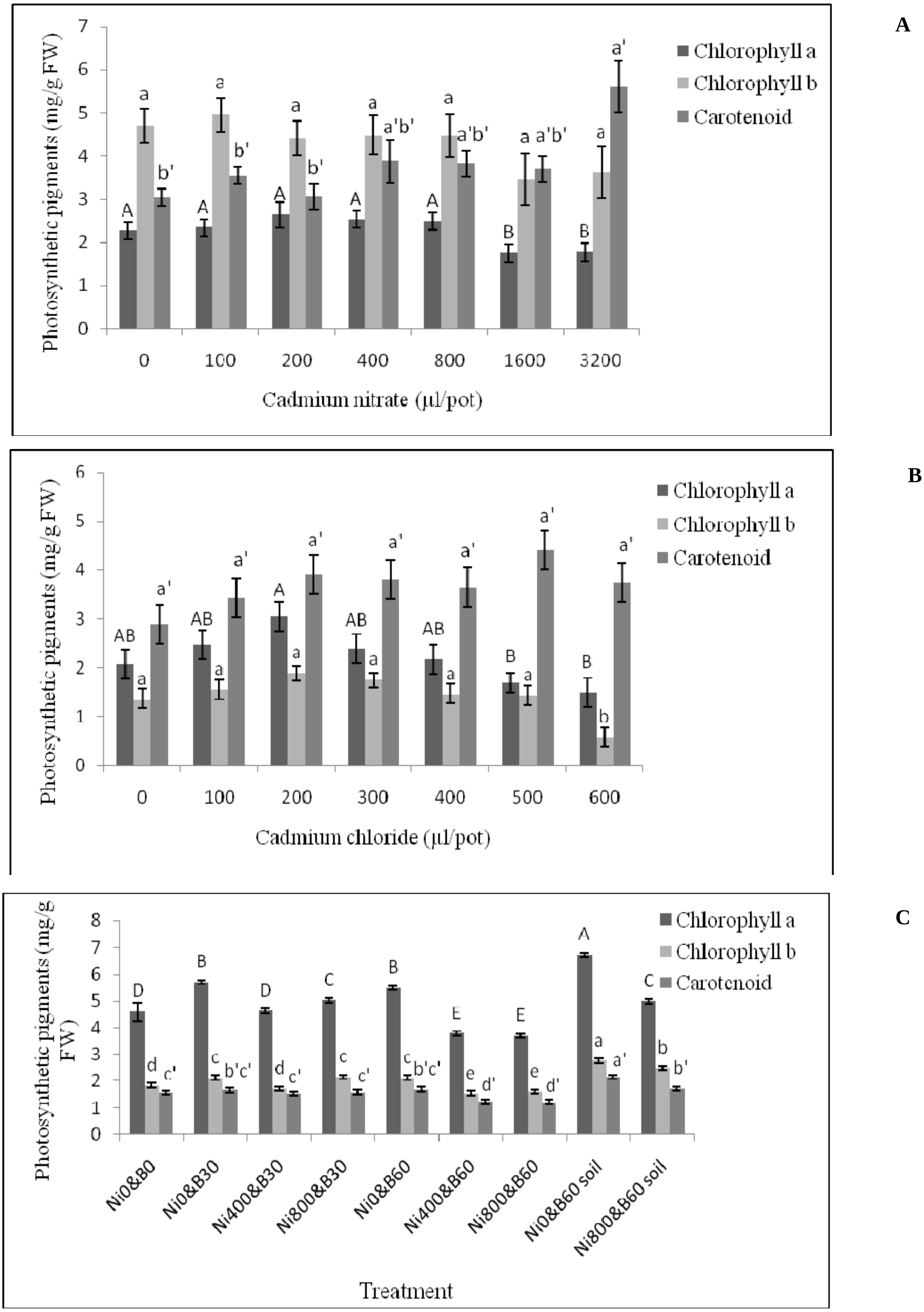

Fig 2 Effect of cadmium nitrate (0, 100, 200, 400, 800, 1600 and 3200 µl from 100µM stock) (A), cadmium chloride (0, 100, 200, 300, 400, 500 and 600 µl from 100µM stock) (B) and nickel chloride (400 and 800 µM) in the presence of 30 and 60 ml from commercial stock of vitamin B complex (C) on *A. vera* L. seedling leaves pigments. Different small letters and different capital letters show significant differences between means at $P \leq 0.05$.

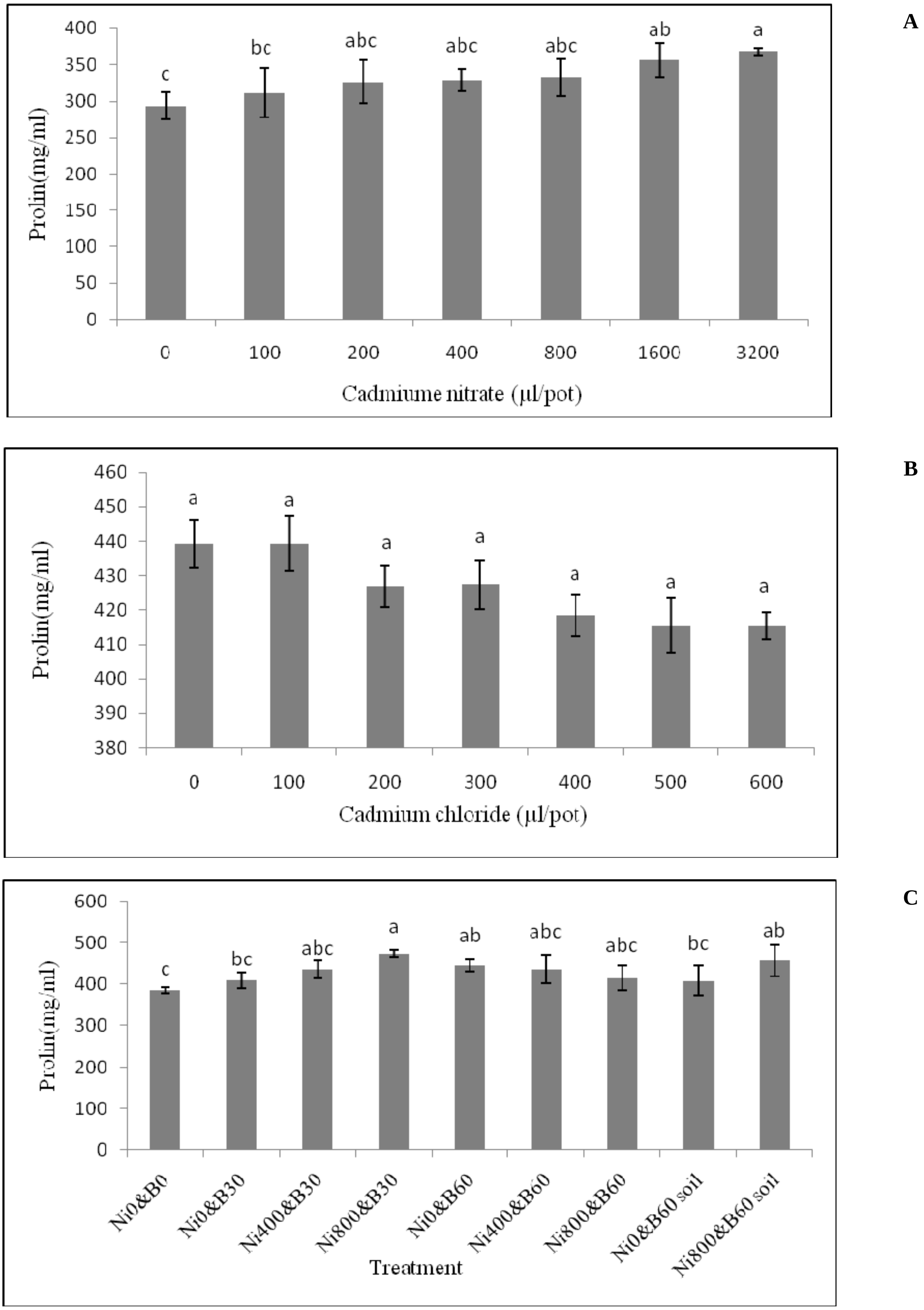

Fig 3 Effect of cadmium nitrate (0, 100, 200, 400, 800, 1600 and 3200 µl from 100µM stock) (A), cadmium chloride (0, 100, 200, 300, 400, 500 and 600 µl from 100µM stock) (B) and nickel chloride (400 and 800 µM) in the presence of 30 and 60 ml from commercial stock of vitamin B complex (C) on *A. vera* L.seedling leaves free proline content. Different small letters show significant differences between means at $P \leq 0.05$.

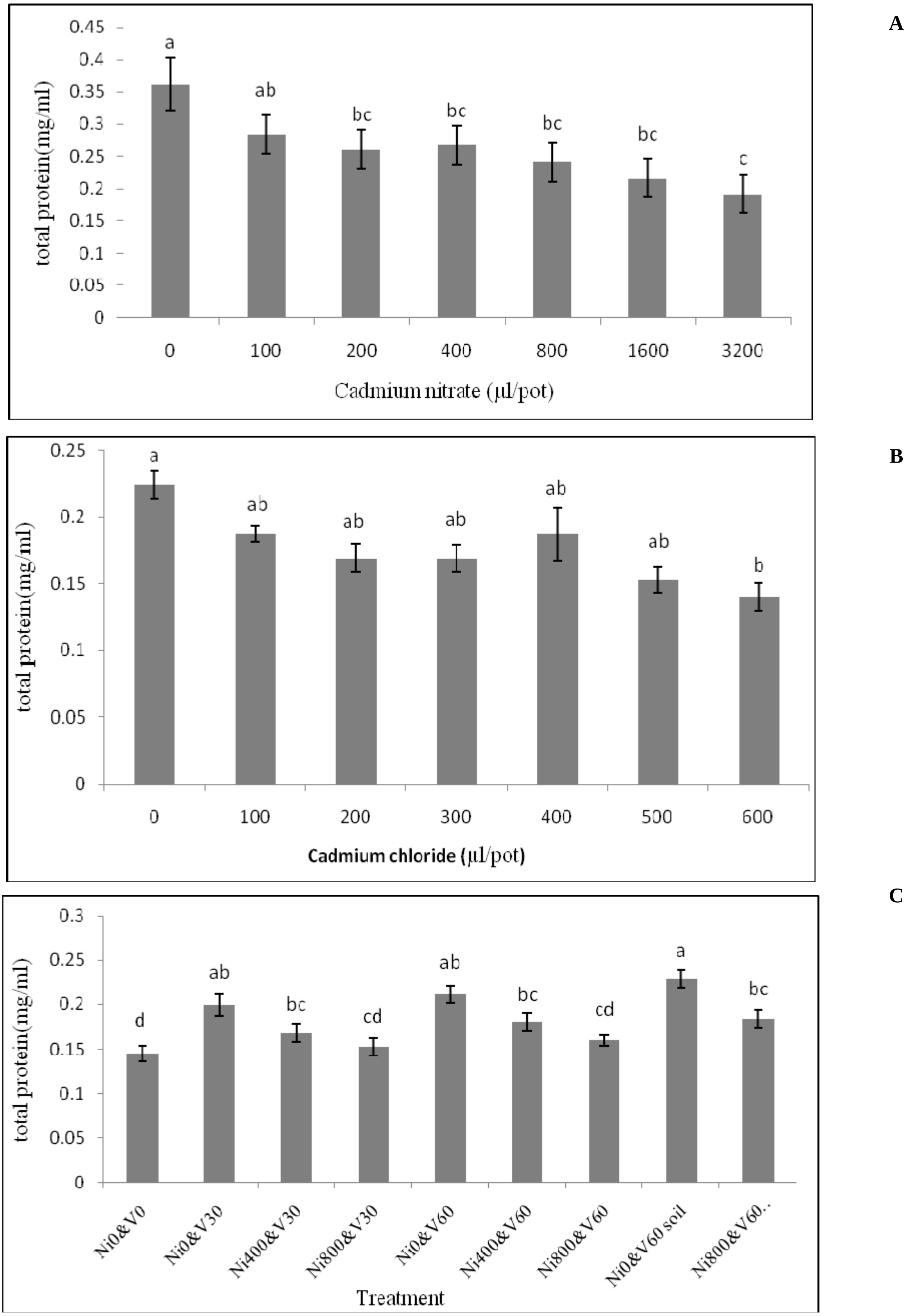

Fig 4 Effect of cadmium nitrate (0, 100, 200, 400, 800, 1600 and 3200 µl from 100µM stock) (A), cadmium chloride (0, 100, 200, 300, 400, 500 and 600 µl from 100µM stock) (B) and nickel chloride (400 and 800 µM) in the presence of 30 and 60 ml from commercial stock of vitamin B complex (C) on *A. vera* L. seedling leaves total protein content. Different small letters show significant differences between means at $P \leq 0.05$.

Total protein content of *A. vera* L. seedling leaves reduced with increasing the amount of cadmium nitrate. High level of cadmium chloride (600 µl from 100µM stock) reduced the total protein content. Nickel chloride (800 µl from 100µM stock) in the presence of 30 and 60 ml from commercial stock of However, it interferes indirectly with a number of antioxidant enzymes [36], for example, superoxide dismutase (SOD), glutathione reductase (GR), peroxidase (POD). Exposure of plants to Ni at low concentrations (0.05 mM) and/or for short time periods have been shown to increase the activities of SOD, POD and GR in order to enhance the activation of other antioxidant defenses and hence lead to the removal (or scavenging) of ROS [37]. However, excess Ni has been found to reduce the activity of many cellular antioxidant enzymes, both *in vitro* and *in vivo*, and plant's capability to scavenge ROS, leading to ROS accumulation and finally oxidative stress in plants [36- 37].

The results showed that vitamin B complex has a positive effect on growth and some biochemical parameters and its applications in the soil is comparatively better than spraying on the leaf. It may be related to its stability in the soil. Denslow and co-workers reported that exogenously applied vitamin B_6 protects plant cells against cell death induced by singlet oxygen. These results showed that plants employ vitamin B_6 as an antioxidant to protect themselves against reactive oxygen species [12]. This study must be repeated on other plants with vitamin B complex and or with different kind of B vitamin (B_1 to B_{12}) on *A. vera* L.. The vitamin B complex may ably help plant's stress tolerance.

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