Effects of Nickel Salt Concentrations on Germination and Development of *Grevillea exul* var. *rubiginosa*

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Abstract

Endemic plant species have been considered as one of the best means of ecological restoration of exploited mine sites in New Caledonia. These plants have the adaptability characteristics that allow them to thrive on serpentine soils. This study has investigated the physiological effects of nickel concentrations at different developmental stages on Grevillea exul var. rubiginosa an endemic New Caledonian species. The first two stages of a plant life cycle germination and post germination under controlled conditions were studied. The experiment involved Petri dish germination and growth of seeds and seedlings respectively particularly root development and length in different salts of nickel (acetate, chloride and sulphate) with 0 – 500 ppm concentrations. Decrease in both studied parameters: germination rates and root lengths were observed with increasing concentrations of nickel solutions supplied to seeds. A minimum concentration of 5 ppm of nickel in the medium had a positive effect on germination rate as well as root length. Results suggest that it is the concentration of nickel used in the present investigation to test the germination rate and root elongation in Grevillea exul var. rubiginosa which are essential for experimental work in the field rather than the form (salt) of nickel. These results will be help-full in re-vegetation efforts of nickel mining sites in New Caledonia.

Key Words: *Germination rate, Root lengths, Serpentine soils, Physiological effects.*

1. Introduction

New Caledonia, a South Pacific archipelago has a land area of 19000 km², spread over an area of 500 km long and 50 km wide. Approximately one third of the total surface area of the island 5500 km² is covered with serpentine soils (International Nickel Study Group, 2001; McCoy *et al*., 2003; Sarrailh and Ayrault, 2003). These soils are derived from ultramafic rocks, which are very rich in metals (nickel, cobalt, manganese and chromium) but poor in plant nutrients (calcium, potassium, phosphorus and nitrogen) as well as organic matter.

New Caledonia is the third largest producer of nickel in the world, mining of nickel began around 1876 and the main form of mining was carried out in open pit mines. Intensive mining activities became a concern to the people when pollution began to affect their normal way of life. Dumping of mine wastes into surrounding valleys caused sedimentation in coastal rivers and destruction of fringing reefs (Bird *et al*., 1984). These situations generated increasing environmental awareness for the need to revegetate mine sites and to develop new mining techniques in order to reduce the harmful environmental impact (Pelletier and Esterle, 1995; McCoy *et al*., 2003; Sarrailh & Ayrault, 2003). In New Caledonia, 90 – 98 % of the plants are endemic (Jaffre, 2003) and recent mine restoration efforts have focused on the diverse endemic ultramafic flora as an important source of species. These species are adapted to extreme substrate and climatic conditions of mine sites (Jaffre *et al*., 1997).

Plant development is a continuous process and seed germination is the beginning of this process. Seeds are a convenient source to begin with since they are quiescent, resting entities that represent a normal hiatus in the life cycle. Seeds are severely dehydrated. Their water content is normally about 5 % or less (Hopkins, 1995) and metabolic reactions are scarcely detectable because these reactions take place very slowly. Hence seeds can be stated to be in a state of suspended animation, and are capable of surviving adverse conditions for long periods of time without growing. When conditions are appropriate, the embryo renews its growth and the seeds germinate.

This study focuses on an endemic plant species of New Caledonia *Grevillea exul* var. *rubiginosa*. This species is adapted to the extreme and hostile habitats of serpentine soils.

These soils have high concentrations of heavy metals, which generally has an adverse effect on plant growth (Westerbergh, 1994), low concentrations of calcium in comparison to magnesium (Proctor, 1971). These soils are dry and exposed because of the granular texture and lack of organic material (Brooks, 1987). The distinctive features of this species are broad leaves with reddish undersides, growth to a maturity of 5 – 10 meters and its ability to retain unstable rocks. Due to the importance of *Grevillea exul* var. *rubiginosa* for revegetation, it was thought necessary to study the physiological aspects in order to determine effective means of germination, growth and revegetation of exploited mine sites, which contains high nickel salt concentrations.

2. Materials and Methods

Grevillea exul var. *rubiginosa* is a woody species and belongs to the family Proteceae, common in New Caledonia. It produces fruits once a year during the summer months (October – March), fruits have a hard covering that protect the two seeds inside. For the present investigation fruits were collected from the Southern province in February 2003 and left under artificial light to dry until the seeds inside were released (~1 week). Seeds were stored in airtight storage bottles at - 4 °C for later use.

3.2 Controlled Germination

To determine seed germination percentage, *Grevillea exul* var. *rubiginosa* seeds were washed with 4% sodium hypochlorite solution for 30 minutes once, then four times for 5 minutes each with sterile water before being sowed in 8 mm Petri dishes lined with 3 layers of Whatman filter paper moistened with 8 ml of nickel solutions (0 – 500 ppm concentrations). All Petri dishes were sealed once they were set up. Three salts of nickel were used: nickel acetate, nickel chloride and nickel sulphate. Sterile conditions were maintained at all times to minimize the risks of contamination by working in the laminar flow.

Germination was allowed to occur in a dark incubator at 30 °C, the following germination conditions were maintained: a temperature of 30 °C, darkness, 1 ml of nutrient solution per cm Petri dish, because they were the most appropriate for the species according to Léon (V. Leon, PhD student, Laboratoire de Biologie et Physiologie Vegetale Appliquees, UNC, personal communication). Number of germinated seeds and root lengths were recorded twice a week until the germination rate became constant (~1 month). Each treatment had 3 replicates and was repeated twice. Germination rate (%) for each treatment was calculated as follows: (no. of germinated seeds/total no. of seeds) x 100. Germination rate and root lengths were graphed to determine if a correlation existed between the two factors. Graphpad prism 4 statistical package and Microsoft excel programmes were used for data analysis. Two – way ANOVA was conducted to determine the interaction effect of GR and RL's with nickel concentration (ppm) and the three nickel salts.

3. Results

Results obtained on seed germination, root: shoot growth and development and various other responses by New Caledonian nickel tolerant endemic plant *Grevillea exul* var. *rubiginosa* are presented in this paper.

3.2 Germination Rate and Root Lengths

Germination rates (GR) and root lengths (RL) were the two major indicators of the environment and nutrient effect on seeds during germination. Nickel chloride showed the usual response of high concentrations of nickel to *Grevillea exul* var. *rubiginosa*. The response being positive at 5 ppm and 10 ppm for GR as well as RL, whereas a decrease and finally inhibitory effect sets in with increasing concentrations of nickel chloride 50 ppm, 100 ppm and 500 ppm. Germination rates for nickel acetate were highest at 50 ppm, 5 ppm than 10 ppm with 100 ppm having the lowest GR. Nickel chloride and sulphate followed a similar trend with the highest GR at 5 ppm and 10 ppm then decreased dramatically at 50, 100 and 500 ppm for nickel chloride. Whereas for nickel sulphate GR remains constant for 5, 10 and 50 ppm but decreased at 100 ppm and the lowest GR was recorded in the highest (500 ppm) concentration of nickel as indicated in Figure 1. At 50 and 100 ppm nickel acetate concentrations an increase in root lengths was noted as seen in Figure 2 as compared to 0, 5 and 10 ppm concentrations.

3.2 Nickel chloride

For nickel chloride the rate of germination over time (0 – 44 days) was highest in 5 ppm increasing gradually reaching a maximum of 80 % on the 28th day and remained constant until treatment was terminated. A similar pattern was observed in 0 ppm and 10 ppm with 10 ppm having a higher GR which became constant at 66 % on the 24th day and finally increased to 80 % on the final (44th) day of observation as seen in Figure 3. For control (0 ppm) GR gradually increased reaching 73.3 % on the final day (44th) of recording. At 50 ppm and higher concentrations the rate of germination decreased rapidly with 500 ppm concentration of nickel inhibiting germination almost completely. A similar pattern was observed for root lengths over time with 5 ppm of nickel, which had the longest roots followed by 10 ppm and control until the 32nd day. Treatment with concentration of 10 ppm nickel had the longest roots of 92 mm (Figure 4), followed by control (83 mm) and 5 ppm treatment (78 mm) on the last day of recording. Root lengths begun to decrease at 50 ppm, the lowest observed at 100 ppm. An inhibitory effect at 500 ppm concentration was recorded.

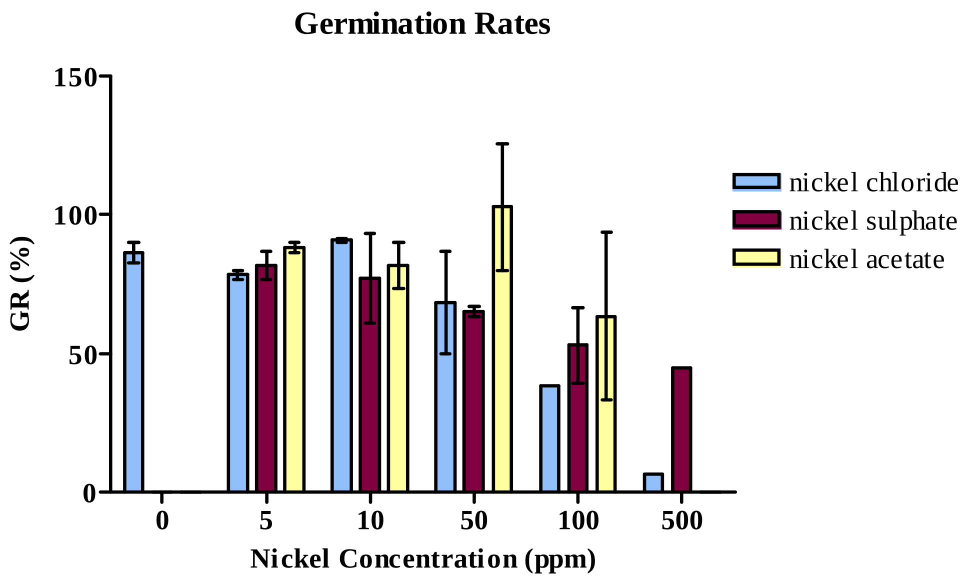

Figure 1. Seedling germination percentage of Grevillea exul var. rubiginosa in 3 nickel salt concentrations.

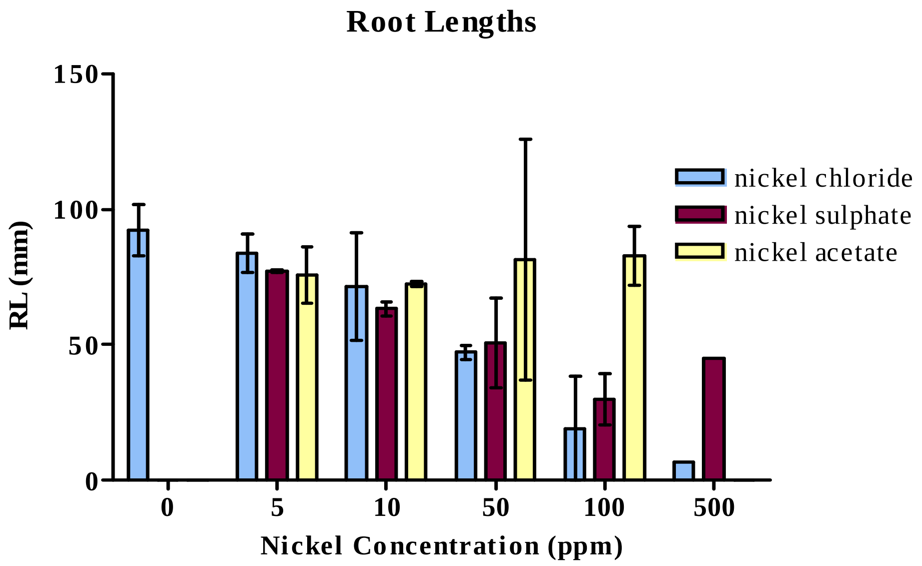

Figure 2. Seedling root lengths of 3 Grevillea exul var. rubiginosa in 3 nickel salts concentrations.

3.3 Nickel sulphate

Nickel sulphate concentration on GR did not show differences between control (0 ppm), 5, 10 and 50 ppm nickel concentrations but a decrease was noted for 100 and 500 ppm indicating the inhibitory effects of nickel at higher concentrations. As compared to control, GR was better in 5, 10, and 50 ppm after 28th day reaching a maximum 73.3 % (Figure 5). The germination rate at 500 ppm in nickel sulphate showed lower inhibition rate as compared to nickel chloride. Germination in nickel sulphate begun after 16th day and continued to increase reaching a maximum of 23.3 % on 44th (final) day, whereas germination in nickel chloride was delayed until 26th day, increased to 3.3% and remained constant until treatment was terminated.

Root length elongation showed similar trend as GR in 0 ppm) control, 5, 10, 50 and 100 ppm (Figure 6). Root length was almost same in all these concentrations until 20th day. Root lengths in 50 ppm nickel concentration begun to increase as compared with control and 10 ppm concentrations. Finally 50 ppm concentration treatment had longer roots on 32nd day as compared to 10 ppm. A similar pattern was recorded in 100 ppm and 500 ppm with the greatest inhibitory effect in 500 ppm nickel concentration. This inhibitory effect became less obvious by 32nd day when root lengths increased dramatically in comparison to 100 ppm.

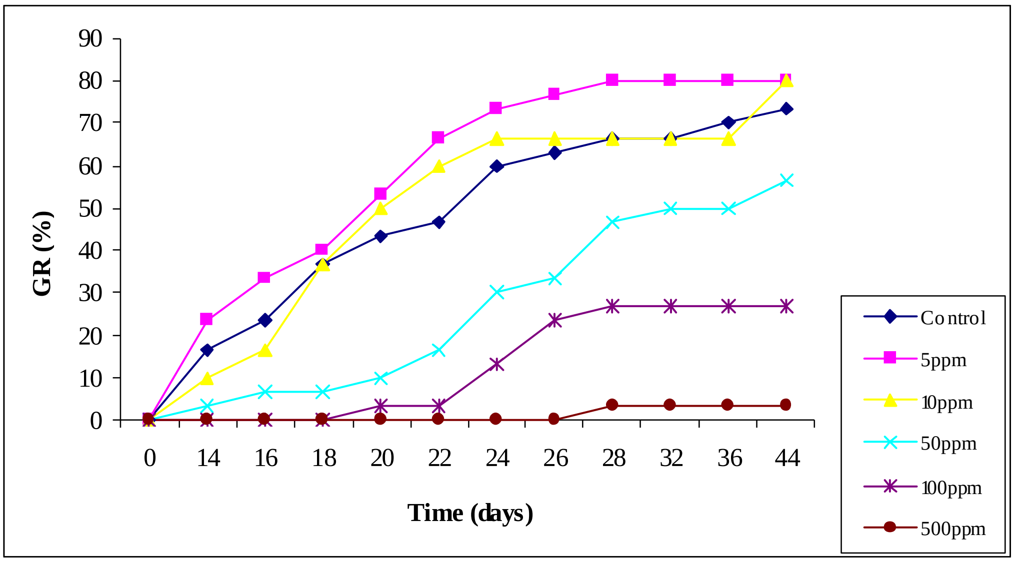

Figure 3. Seed Germination rate in nickel chloride germinated seedlings.

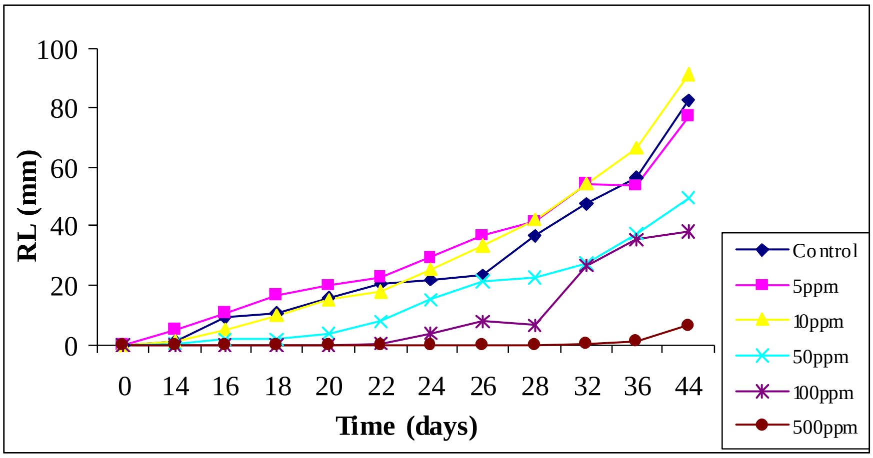

Figure 4. Root Lengths of germinated seedlings in nickel chloride.

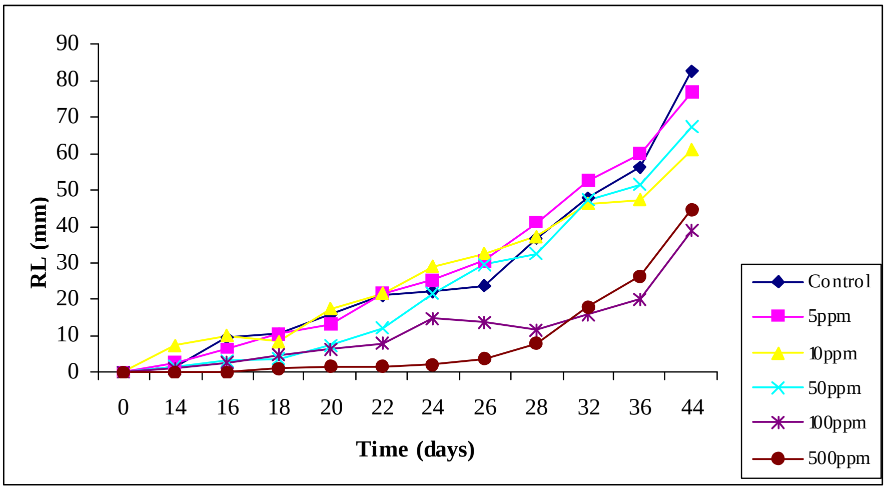

Figure 5. Seed germination rate in nickel sulphate germinated seedlings.

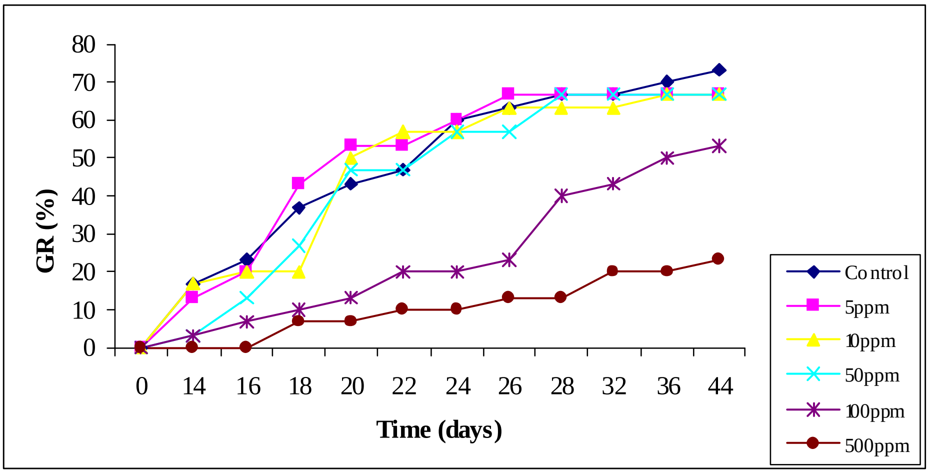

Figure 6. Root Lengths of germinated seedlings in nickel sulphate**.**

3.4 Nickel acetate

Nickel acetate was the nickel salt that showed inconsistent pattern as compared to nickel chloride and nickel sulphate. Nickel acetate concentrations of 5 ppm had the highest GR of 93.3 % followed by 10 ppm at 90 % as seen in figure 7. No major differences were observed between control (0 ppm) and 50 ppm between 18 and 24 days. Initially control (0 ppm) had a higher GR until the 16th day after which GR in 50 ppm was better than control. After 24th day with a GR of 60 %, a gradual increase was observed for control which reached a maximum of 73.3 %, and ended up being higher than in 50 ppm concentration. Germination rate in control (0 ppm) increased as compared to 50 ppm, which remained constant by 24 to 36 day then increased 10 % as compared to control (0 ppm) by the final (44th) day.

Germination rate in 100 ppm nickel acetate was similar but lower than 50 ppm concentration of nickel for 20th day after which GR increased by 10 % on the 22nd day. There was no further increase till 24th day then increased to 80 %

by 28th day and then remained constant for the next 8 days and finally reached a maximum of 83.3 %.

As seen in figure 8 root lengths followed a similar pattern (inconsistent) for all concentrations where 50 ppm nickel concentration showed enhanced root lengths from 22nd day onward compared to all other concentrations. Finally root length reached a maximum of 125.6 mm for 50 ppm nickel, which ended up being the longest roots for all compared concentrations of 3 nickel salts followed by 93.5 mm root length in 100 ppm nickel acetate.

4. Discussion

Although seed germination tests were used to determine better and quick germination and seedling response (Archambault and Winterhalder, 1995), environmental factors also affect the pattern and duration of germination depending on the interaction between the internal factors of the seed and the environment (Karataglis, 1980). Results obtained in this investigation showed that germination rates and root lengths are affected to a large extent by high nickel concentration in the germinating medium. Germination and growth were inhibited at very high concentrations of nickel salts. This also included delayed germination and poor establishment of seedlings with increasing nickel concentrations.

According to the results obtained for germination rate the different salts of nickel are not considered significant ($P = 0.5773$) whereas the various concentrations of nickel (ppm) are considered extremely significant ($P < 0.0001$). A similar pattern is also observed for root lengths where the different salts have no significant effect overall ($P=0.3039$) whereas the concentration of nickel (ppm) is considered to be extremely significant ($P = 0.0002$). Overall interaction between the salts and and if they have the same effect at all values of nickel concentration (ppm) is considered to be very significant for GR ($P=0.0052$) and RL ($P=0.0062$). Results similar to the present study but in different plant (Bilberry seedlings) showed that nickel ions at higher concentrations suppressed seedling growth by inhibiting cell expansion and division (Lyanguzova, 1999). Hence the concentration of nickel (ppm) used is a factor that needs to be taken into consideration rather than the form of the nickel salt when trying to determine the effect of nickel on germination and root length of *Grevillea exul* var. *rubiginosa*.

Peralta *et al.* (2001) also reported the effects of nickel and other heavy metals such as Cd, Cr, Cu and Zn on seed germination, root and shoot elongation as metal concentration in the growing media was increased. Calabrese and Baldwin (1999) reported the phenomenon

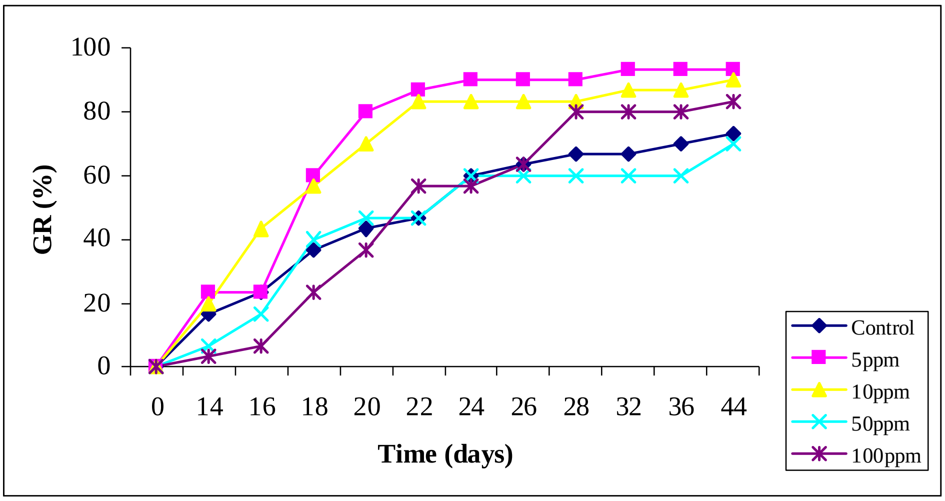

Figure 7. Seed germination rate in nickel acetate germinated seedlings.

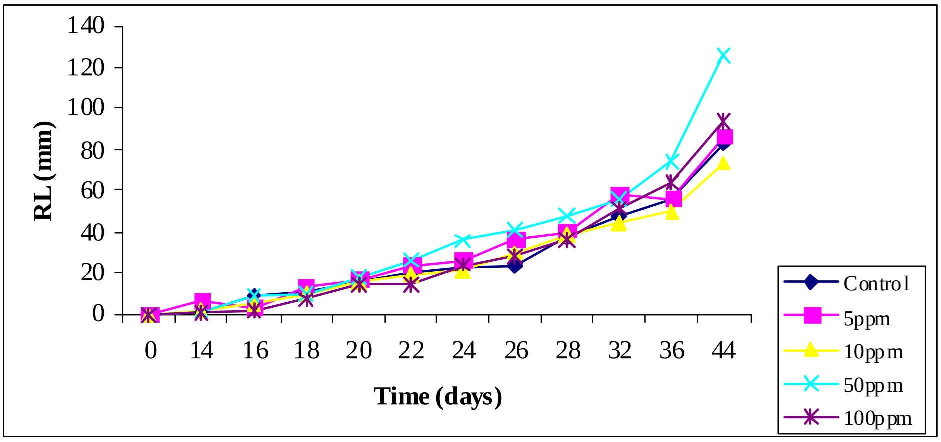

Figure 8. Root lengths of germinated seedlings in nickel acetate.

known as hormesis, where smaller doses of heavy metals increase seedling growth while greater doses decrease the seedling growth.

The effects of the environment on germination are quiet complex because of interactions and internal factors that modify germination patterns (Rtout *et al.*, 2000). According to Hilhorst and Kerseen (2000) three environmental factors that can be accurately sensed by seeds are light, nitrate and temperature (fluctutations).

From the results obtained in the present investigation it is quite obvious that nickel is essential for better germination and growth of *Grevillea exul* var. *rubiginosa*. Five ppm concentration of nickel is considered beneficial for plantlets, since this was the concentration of nickel in the growing medium that had a positive effect on both seed germination and root elongation. However nickel at higher concentrations inhibits cell division and cell expansion in the meristemic zone of the roots (Robertson and Meakin, 1980; L' Huillier *et al.*, 1996; Piccini and Malavolta, 1992).

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