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RESEARCH ARTICLE

EFFECT OF METAL ON GERMINATION AND PROLINE ACCUMULATION IN *SPINACEA OLERACEA*

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ABSTRACT

The presence of heavy metals in the environment is of major concern because of their toxicity, carcinogenicity, mutagenic and bio-accumulating tendency threat to the human life and the environment. Spinach is a popular leafy vegetable, rich in proteins and vitamins. It has been reported earlier that Spinach accumulate nitrates and oxalates in the leaves in response to sewage water irrigation. In the present investigation, *Spinacea oleracea* "variety all green" was grown, irrigated with different ppm solutions (50, 100, 300, and 500) of Co, Zn and Pb salt each to study the effect on the germination percentage and germination index of the seed. The germination index was greater in 500ppm concentration in all salt solution used ($CoSO_4$, $ZnSO_4$ and $(CH_3COO)_2$ Pb), though in lower concentrations (300,100,50 ppm) it was consistent and no variations were observed . Among the three salt solutions used for irrigation, response of the seeds to Pb showed least germination percentage. The proline content in the experimental plants was investigated to evaluate the stress response. In plants irrigated with Pb solution the proline content was found to be very high as compared to other salts ($CoSO_4$ and $ZnSO_4$).

Key words: $CoSO_4$, $(CH_3COO)_2$ Pb, germination percentage, germination index, proline, Spinach, $ZnSO_4$

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INTRODUCTION

Modern industry is to a larger extent responsible for the contamination of the environment over the past decade and the consumption of metals and chemicals in the processing industries has increased dramatically posing threat to the human life and the environment (Malik A., 2003). Heavy metals are among the conservative pollutants that are not subject to the bacterial attack or other break down processes and are therefore permanent additions to the environment (Duruibe *et al*, 2007). Heavy metals might accumulate in the food chains, with the risk for the health of animals and humans which are less sensitive to metals toxicity than plants (Parmar *et al.,* 2002*).* Most plants are sensitive to heavy metals in excess of minimal concentrations. However, certain plants species can grow on contaminated habitats because they have developed a variety of avoidance mechanism by which the excess of heavy metals can be rendered harmless. Heavy metal pollution is of anthropogenic origin so in future their intensity is likely to increase, therefore study of plants exposure to heavy metals particularly at biochemical level deserve priority (Handique and Handique, 2007). Metal ions are essential in low concentrations for the maintenance and development of all organisms.

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Many molecules and reactions in plants require essential elements. Macro elements function include Magnesium for functional chlorophyll, activation of ATP synthase, enzyme RuBPCO; Iron for electron transporters or catalysis and Calcium for signaling photo activation of Photosystem-II. Micro elements are involved in various functions, Manganese for oxygen evolving complex; Copper for plastocyanin and Cobalt for vitamin B_{12}. However, metals present in excessive amounts may interfere with cellular metabolism and become toxic (Kucera *et al,* 2008). The most toxic to higher plants and microorganisms even at low concentrations are the nonessential metal ions such as Cd^{2+},Hg^{2+}, As^{3+5+}, Pb^{2+4+}, Ag^{+}, Cr^{6+} *etc*. The present investigation was undertaken to study the effect of three selected metals *viz* Co (essential component of several enzymes and co-enzymes), Zn (essential micronutrient involved in a wide variety of physiological processes) and Pb (nonessential metal), in different concentration on the germination and proline accumulation in *Spinacea oleracea*.

MATERIALS AND METHODS

Spinacea oleracea "variety all green" seeds used for the study were procured from a government recognized nursery in Mumbai. For the study of germination percentage and germination index, the seeds were first surface sterilized in 0.5% sodium hypochlorite solution, washed thoroughly with distilled water and then laid for germination in petridishes (diameter- 15 cm) with double layers of Whatmann filter paper soaked in fixed volume of distilled water (control), 50ppm, 100 ppm, 300 ppm and 500 ppm solutions each of different salts like Zinc Sulphate, Cobalt Sulphate and Lead Acetate. The petridishes were kept in dark at 30 ±1°C. Three replicates of 15 seeds each were kept for observation. Germination was recorded daily at fixed hour and the emergence of the radical was taken as the criterion of germination. The root and shoot length were measured and the germination percentage values were calculated. Also, the seeds were later sown in pots (15cm diameter x 20cm height) filled with fertile loamy soil, irrigated initially with tap water for 7 days and then with different ppm solutions (50, 100, 300, 500) of Co, Zn, Pb salts and tap water as control. The leaves were harvested on the 7th, 14th, 21st and 28th days for the estimation of proline (Bates *et al*, 1973) and chlorophyll (Arnon, 1949). All the experiments were carried in triplicates, repeated once, than the results were averaged and the data obtained were statistically analyzed.

RESULTS AND DISCUSSION

In recent decades, the use of sewage water to grow leafy vegetables is considered as an efficient way to reuse water. Though, due to sewage water irrigation the leafy vegetable spinach is loaded with nitrates and oxalate making it unhealthy for human consumption (G. Nair and Hippalgaonkar, 1996). Moreover the study of stress has gained increasing importance in many fields of biology and a wide variety of approaches have been employed in the attempt to gain more insight into the process involved. The data obtained for the germination studies using three different salt solutions (Fig-1) indicated that increasing concentration of Co and Zn metal had positive influence on the germination in seeds of *S. oleracea* plant. Irrespective of the salt or the various concentrations used, no failure in germination was observed. Pb irrigation adversely affected the germination in spinach; there was 25-30% decrease in germination percentage than that observed in Co, Zn treated seeds and also to that in control. Moreover, in seeds irrigated with Pb (lead acetate) the sign of germination appeared to be delayed or time taken to germinate was more and was observed only on the 12 DAS as compared to the other treatments where germination of the seeds were observed on the 6 DAS.

It can be implied that Pb (lead acetate solution) as a potentially toxic heavy metal with no known biological function posed some kind of inhibitory effect on the germination of Spinach seed. The response of *Spinacea oleracea* seeds was emphatic in all concentrations of cobalt sulphate treatments. The germination percentage in 50 and 100 ppm was similar to that as in the control, but it increased subsequently in response to higher concentrations of 300 and 500 ppm suggesting a stimulating influence of Co salt on the germination percentage. Moreover, Spinach seeds germinated in all concentrations of zinc sulphate treatments though at lower concentrations (50 ppm, 100 ppm) the germination percentage almost reduced to half than that of the control (tap water) and there was a corresponding increase of almost 1.5 and 2.0 times more than the control at higher concentrations 300 ppm and 500 ppm respectively. The highest percentage of germination was observed in 500 ppm of zinc sulphate (80 ± 3.6%) as compared to cobalt sulphate, lead acetate and control which indicates that Spinach seeds can tolerate high Zn concentration as compared to other salt solutions (cobalt and lead). It appears that the reduction in germination at the lower concentration of $ZnSO_4$ may be due a kind of initial shock reciprocal to the load of $ZnSO_4$ concentration, whereas indicating that high doses of zinc may in some way supports the germination process. A one–way ANOVA revealed that $ZnSO_4$ treatments was highly significant ($P< 0.01$; $F= 8.5$) as compared to other salt solutions of Co and Pb .The treatment of Co salt solution was also significant though at $P< 0.25$ ($F=4.5$) while the influence of Pb salt solution though was obvious and was insignificant. The germination index was highest in 500ppm of all the salt solutions (cobalt, zinc, lead), though it was consistently similar in the lower concentrations (300ppm,100ppm,50ppm).The data of germination index at 500 ppm in decreasing order can be expressed as follows: $CoSO_4$ (4.79± 1.07)>$ZnSO_4$ (2.97±1.03) >control (2.09 ± 1.09) >$(CH3COO)_2Pb$ (1±1.02). The shoot (coleoptiles) length was greater than root length in all the salt solutions treatment, however in the control samples no such difference were noted and both measured 0.15cm each. Moreover, the shoot and root length growth decreased significantly in correlation to the concentration of all the metals as compared to the respective control samples and was the least in highest concentration of all three metals. Radicle and plumule growth are the primary plant organs that sense, contact and accumulate heavy metals from the substrate and are thought to be a reliable indicator of metals tolerance in plants (Patra *et al*, 2004; Ozdener and Kutbay, 2009). Studies by some scientist (Ling *et al,* 2010) on the effect of mercury on four vegetable crops have also reported a significant negative correlation between the root length and mercury concentration. In this study, we have observed diverse response by the spinach seeds to different metals in varying concentrations.

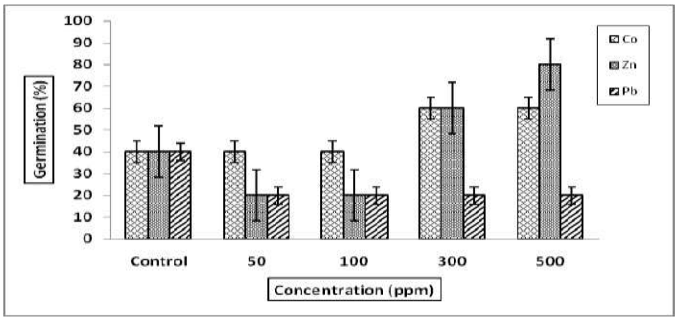

Fig 1. Effect of Different salt Solution on Germination %

Proline accumulation in the *Spinacea oleracea* test plants as a function of different metal concentration in a range between 0 – 500 ppm in the soil was also studied in the leaves collected from plants irrigated with different salt solutions (Co, Zn, Pb) on the 7th ,14th ,21st and 28th day after sowing (DAS). The proline content was calculated micro mol per gram and is presented in Fig-2a, 2b and 2c. It is evident from the results that exposure to either metal (Co/Zn/Pb) in increasing concentration resulted in sharp rise in the proline content in the test plants. Proline accumulation was of greater magnitude in the test plants especially at 500ppm concentration in all salt solutions, though in Lead acetate the increase was much evident and maximum (30 ±3.4 %) than the other treatments. It was very obvious that proline accumulation was negligible in the leaves from plants of 7 DAS irrigated with tap water. Subsequently there was a progressive increase in the proline content up to the 28th day in the order 50 ppm <100ppm< 300 ppm <500pm. Heavy metal stress leads to proline accumulation[11]. Proline accumulation, accepted as an indicator of environmental stress, is also considered to have important protective roles. From the present data, variation in proline accumulation in response to metal concentration was apparent;

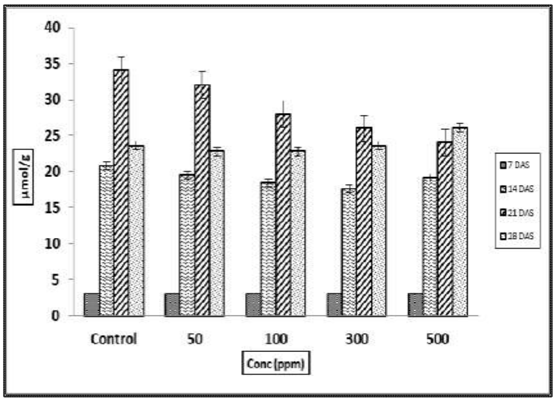

Fig. 2a.

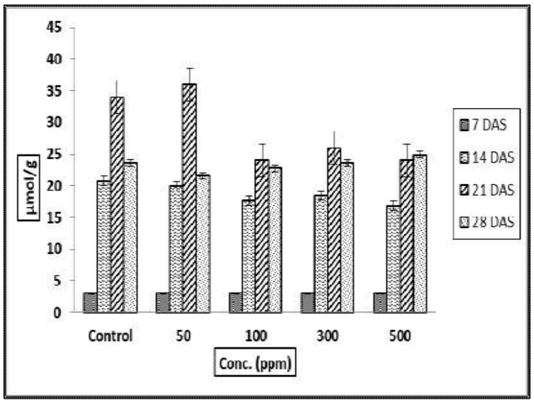

Fig. 2b.

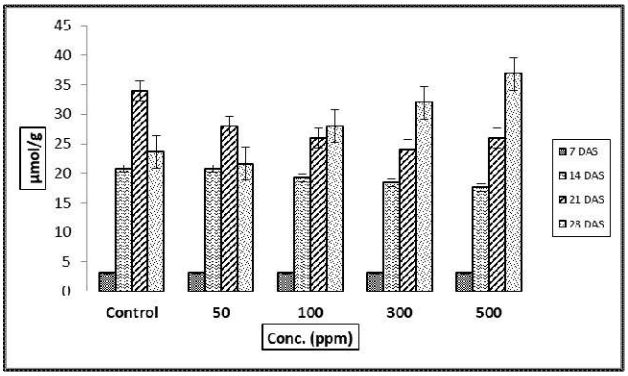

Fig. 2c.

Fig. 2. Effect of different concentration of a) CoSO4 b) ZnSO4 c) (CH3COO)2Pb on Proline content in *Spinacea oleracea*

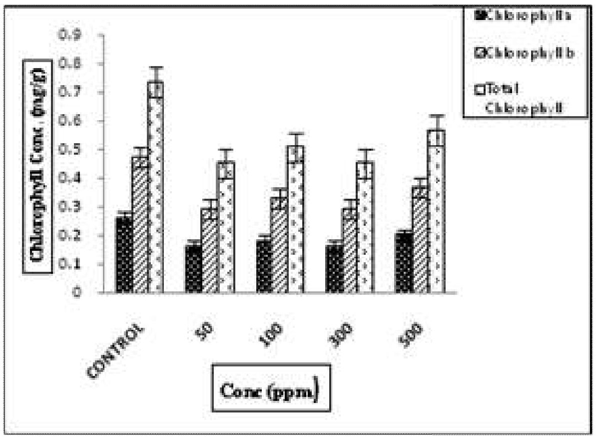

Fig. 3a.

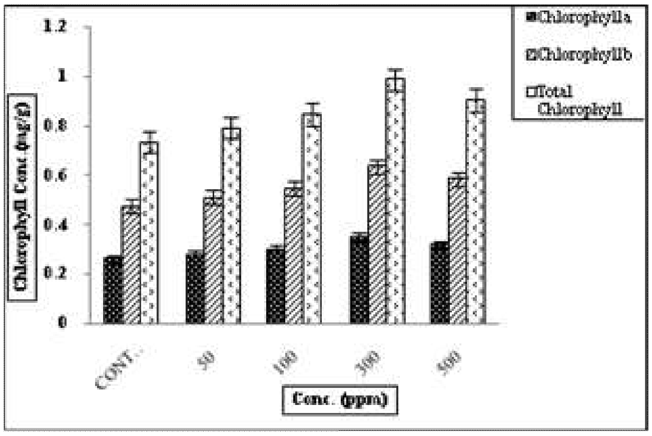

Fig 3b.

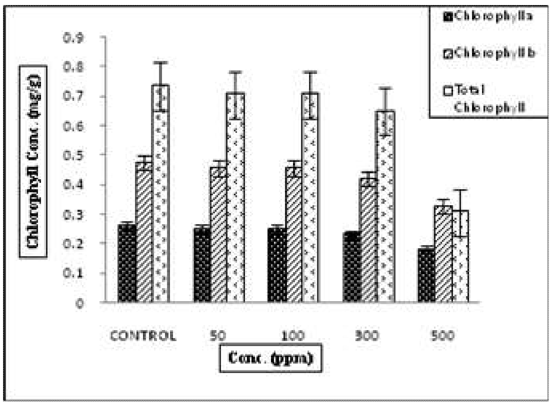

Fig. 3c.

also it seemed to be metal specific. The metal induced proline accumulation was found to be dependent upon the duration of exposure, though the variation pattern was different for the three metals with Pb exposure recording highest proline content as compared to the others. There are evidences that support the fact that abiotic largely stress like toxic heavy metal stress has induced proline accumulation in mung bean (Roy and Bera, 2003), in peas (Metwally *et al*, 208) and in tomato (De and Mukherjee, 1998). The present findings holds true with the earlier report that heavy metal induced proline accumulation can be used as biochemical indicator of heavy metals pollution (Parekh *et al*, 1990). The exact mechanism of how proline accumulation helps the plant to cope up with heavy metal stress is difficult to elucidate. However, the available evidences suggest that proline acts by protecting the key enzymes from being inactivated by toxic metal ions. Total chlorophyll content declined progressively with increasing concentrations of the lead acetate but the results were consistent in different concentrations of cobalt sulphate where neither any progressive increase nor decrease in chlorophyll content was observed. As compared to the control, the total chlorophyll content was found less in all the concentrations of cobalt sulphate and lead acetate treatment while in plants grown with zinc sulphate irrigation, chlorophyll content was found to be higher and showed progressive increase till 300ppm, in the order 50 ppm <100 ppm <300ppm though in 500 ppm it showed a decline which apparently indicate that high concentration of zinc affected the chlorophyll content (Fig-3a, 3b and 3c). Zinc being an essential micro nutrient influences the chlorophyll content and it is often measured in plants in order to access the impact of environmental stress as changes in pigment content are linked to visual symptoms of plant illness and photosynthetic productivity (Alia P., 1991). Photosynthesis is strongly affected in plants exposed to heavy metals excess (Prasad, 1999).

Researchers have reported decreased chlorophyll in several different plant species under the impact of heavy metals. In two wheat varieties to which Cd and Pb were applied, total chlorophyll decreased by 50% (in *Triticum aestivum* var.*cv Greek* 79) and 70% (in var. *Bolal* 2973) respectively (Oncel *et al*, 2000). Heavy metals inhibit metabolic processes by inhibiting the action of enzymes and this may be the most important cause of inhibition. The decreased chlorophyll content associated with heavy metal stress may be the result of inhibition of the enzymes responsible for chlorophyll biosynthesis. Cadmium was reported to affect chlorophyll biosynthesis and inhibit proto chlorophyll reductase and aminolevulinic acid (ALA) synthesis (Stobart *et al*, 1985). It has been reported that Co affects growth and metabolism of plants, in different degrees, depending on the concentration and status of cobalt in rhizosphere and soil (Palit *et al,* 1994). The chlorophyll ratio, which is used as a stress indicator, increased minimally. Earlier work by one of the researcher (Delfine *et al.,* 1999) have reported increased chlorophyll ratio in spinach due to environmental stress. It has been reported earlier that Lead (Pb) strongly affect the seed morphology and physiology, inhibiting the germination, root elongation, seedling development, plant growth, transpiration, chlorophyll production and water & protein content, causing alterations in chloroplast, obstructing electron transport chain, inhibition of Calvin cycle enzymes, impaired uptake of essential elements, Mg and Fe, and induced deficiency of CO_2 due to stomatal closure (Pourrut *et al*., 2011) Although plant defense strategies exist to cope with heavy metal toxicity via reduced uptake into the cell, sequestration into vacuoles by the formation of complexes, binding by phytochelatins, synthesis of osmolytes, activation of various antioxidants to combat ROS, altered expression of enzymes, over expression of genes exist mechanisms by which germinating seeds combat heavy metal stress remains largely unknown (Wang *et al.*, 2011). Moreover it has been suggested by workers that in soils containing high concentrations of Zn, the hyper accumulators species could be cultivated, which would be beneficial for reducing the unusable Zn-polluted fields by the method of phytoremediation. Thus in conclusion it can be perceived that toxic metal ions present in the environment through anthropogenic activity can affect the quality of the plant crops and they represent one of the main a biotic stress factors adversely influencing the plant primarily and the health of man as a secondary effect. Spinach a popular leafy vegetable has been used for the study to assess the response of the crop to the heavy load of metal pollution in the soil existing today. The result obtained and presented implies that Spinach plant is tolerant largely to metal ions Co and Zn and marginally to Pb ions, though the factor like length of exposure time was also important. Hence, use of waste water for irrigating such crop may thereby render the plant unsafe for human consumption which deserves further study.

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