Phytoextraction potential of a weed (*Solanum nigrum* L.) grown in lead contaminated soil

Manjula Thomas[1*], Mayank Varun[2], Rohan D'Souza[1], Manoj S Paul[1]

[1] *Department of Botany, St. John's College, Agra – 282 001, India*

[2] *Department of Botany, Hislop College, Nagpur – 440001, India*

ABSTRACT - This study was aimed to determine the uptake and accumulation potential of a weed (*Solanum nigrum* L.) for phytoremediation of soil contaminated with lead. Plants were grown in soil spiked with 0, 25, 50, 100, 200, 250 mg/kg Pb, individually. Plants sample (root and shoot) were analyzed for Pb content at 25, 50, and 75 days and accumulation trends were characterized. A steady increase in Pb accumulation with increasing metal concentration and exposure period was observed for all treatments. Accumulation of Pb in roots was found to be higher than that of shoots. Statistically significant difference ($P \leq 0.001$) in mean metal content in root and shoot at successive days of study was recorded. Effect of Pb on growth and physiology was also evaluated. At higher Pb levels, root and shoot length, and biomass of test plant were reduced significantly. Although, growth was delayed initially, it was comparable to control at the end of the study. Chlorophyll and proline content declined with the increase in Pb concentration at 25 and 50 days after treatment. However, at 75 days values were more or less comparable to the control values showing the adaptability of test plant in Pb contamination. Considering the moderate accumulation potential, *S. nigrum* could be a potential candidate plant for phytoremediation studies. Hence, phytoremediation employing indigenous weed species like *S. nigrum* can be an ecologically viable option for sustainable and cost-effective management of heavy metal contaminated soils.

Keywords: *Solanum nigrum*, Lead, Phytoremediation, Phytostabilizer

I. INTRODUCTION

The presence of Pb^{2+} and other heavy metals in the environment has become a major threat to plant, animal, and human life due to their toxicity and specially their capacity to bioaccumulate. The possible adverse effects of heavy metal pollution and their phytotoxic effects have been reported by Lee et al. (1998), Paivoke (2002) and Yang et al. (2000). When it accumulates in the human body Pb may cause health problems that include damage to the central nervous system, gastrointestinal tract, cardiovascular system, kidneys as well as hemoglobin biosynthesis, hypertension, and reduced intellectual capabilities. Soil contaminated with lead causes sharp decrease in crop productivity (Johnson and Eaton 1980). Due to such extreme consequences, environmental contamination due to heavy metals, particularly lead is of significant concern. Thus, soil remediation is needed to eliminate risk to humans or the environment from toxic metals like Pb.

Technologies used for removal of heavy metals include reverse-osmosis, ion-exchange, chemical precipitation, solvent extraction, electro-dialysis, adsorption, etc. Most of these technologies are quite costly, energy intensive and metal specific. It has been estimated that the cost of conventional remediating heavy metal-contaminated sites in the USA alone would exceed $7 billion (Salt et al., 1995). Contrary to this, phytoremediation offers a promising technology using plants to remediate or contain contaminants in soil, groundwater, surface water, or sediments (Miretzky et al., 2004). Phytoremediation involves phytoextraction, rhizofiltration, phytostabilization and phytotransformation/phytodegradation. Phytostabilization is one of the strategies of phytoremediation that aims to reduce the mobility and bioavailability of pollutants in environment and not removing them. A significant fraction of metals can be stored within or adsorbed on the root surface contributing to long-term stabilization of pollutants.

The potential of hyperaccumulator plants is often hindered by the slow growth and aboveground biomass, since the majority of the metal hyperacculator plants are poor yielding and slow growing (Salt et al., 1995). Plants suitable for phytoremediation should possess an ability to accumulate the metal, preferably in the aerial parts, tolerance to the metal concentrations accumulated, fast growth with high biomass, and ease of cultivation and harvesting (Baker and Brooks, 1989). However, Chaney et al. (1997) have argued that metal tolerance and hyper accumulation are more important factors than high biomass production.

It has been demonstrated that, wild native plants may be better phytoremediators for waste lands than the known metal hyperaccumulators like *Thlaspi caerulescens* and

Alyssum bertolonii because these are slow growing with shallow root systems and low biomass. Even if the soil is naturally high in a particular metal, native plants often become adapted over time to the locally elevated levels so in the case of native flora and soils, metal toxicity issues, mostly, do not arise.

Globally, the utilization of weeds has been patchy over the past few decades. Nevertheless, there is a renewed interest in focusing on utilization of weeds in productive ways, so that people may benefit from an aspect that has been largely ignored. Weeds are suitable for phytoremediation purpose because of their inherent resistant capability, less cost input and their non-suitability for other purposes like fodder (Lum et al. 2014; Girdhar et al. 2014). *Sesbania*, *Avena*, *Crotalaria*, *Crinum asiaticum* and *Calotropis procera*, lemongrass, vetiver, and other wild grasses have been reported for heavy metal bioindicatoring and phytoremedial purposes (Yang et al 2005; Uraguchi et al. 2006; D'Souza et al. 2010; Varun et al. 2011). Allowing native weedy species to remediate soils is an attractive proposition since a plant community comparable to that existing in the vicinity can be established. The outcome is, thus, both site remediation and ecological restoration.

The present experiment identified a hardy weed plant that could tolerate concentrations of Pb in soil. *Solanum nigrum* (Solanaceae) commonly known as black nightshade is a herbaceous weedy plant with rapid growth, proliferation, strong tolerance to adverse conditions and good biomass production. It was hypothesized that plants with high tolerance could then be tested for their phytoremediation potential. Thus, the aim of the present investigation is (i) to evaluate the phytoremedial potential of *Solanum nigrum* with respect to Pb and, (ii) to study the effect of Pb on its growth and physiology.

II. MATERIALS AND METHODS

2.1. Plant selection - *Solanum nigrum* L.

Commonly known as Black Nightshade belongs to family Solanaceae. It is a short-lived perennial weeds of waste land, old fields, ditches, and roadsides, fence rows, or edges of woods and cultivated land. Annual branched herb up to 120 cm high, with dull dark green leaves, ovate or lanceolate, toothless to slightly toothed on the margins, both surfaces hairy or hairless, petioleolate with a winged upper portion. Flowers small and white recurved when aged and surround prominent bright yellow anthers, pedicellate and five widely spread petals. Fruits small, black when ripe, glossy and raceme. In India, another strain is found with berries that turn red when ripe.

2.2. Experimental design

Solanum nigrum seeds collected from uncontaminated soil were allowed to germinate in a germination tray. After germination, seedlings were transplanted to pots containing 5 kg soil. Single plant per pot was maintained. The seedlings were allowed to grow for 3-4 weeks after which Pb was added to pots at varying concentrations (0, 2.5, 5, 10, 15, 20, 25 mg/kg soil) as aqueous solution using Lead nitrate ($PbNO_3$) salt. Lead nitrate was preferred for its high solubility and subsequent easier availability to the plant. Watering was done as and when necessary with precaution so that the soil remains moist without flooding in order to prevent leaching of lead. A plastic tray was kept below the treatment pot to collect any leachate, which was returned to the pots at next watering. Pots were kept in a random block design. The whole experiment was conducted in green house for 3 months. Any symptoms of metal toxicity exhibited by plants were visually noted. At each sampling date i.e. 25, 50 and 75 Days after treatment (DAT) plants were harvested and taken for metal uptake analysis. Plant growth parameters like root length, shoot length biomass and physiological parameters like total chlorophyll and proline content were also determined.

At each sampling, plants were harvested, washed with tap water to remove adhering soil particles. Samples were further washed thoroughly three to four times with distilled water and finally with de-ionized water and allowed to drip dry completely in a dust-free chamber at room temperature and further used for analysis.

2.3. Pb analysis

For Pb content in soil, 0.5 g soil sample was digested using a wet digestion method with HNO_3 and $HClO_4$ (3:1 ratio) and boiled on a hot plate for 20 min. Plant samples were analyzed by dry ash method where the samples were ashed in a muffle furnace and 0.5 g cooled ash was dissolved in HNO_3 and boiled for 20 min on a hot plate. The filtrate in each case was analyzed for Pb content by Atomic Absorption Spectrophotometer (AAnalyst100, Perkin Elmer, USA), using an air-acetylene flame.

2.4. Biochemical analysis

Chlorophyll content in *S. nigrum* leaf samples was determined on fresh weight basis. 40 mg fresh leaves were placed in 10 ml 80% acetone in a sealed, dark bottle in a refrigerator. After 5 days absorbance of the solution was measured by a spectrophotometer at different wavelengths i.e. 480, 510, 630, 645, 652 and 665 nm and chlorophyll content was calculated using relevant formulae (Arnon 1949).

Amount of proline in plants was determined according to method given by Bates *et al.* (1973). 500 mg of fresh leaves were taken and crushed with 10 ml of 3% sulphosalicylic acid. This was then centrifuged at 3000 rpm for 15 min. 2

ml of supernatant was taken in a test tube and 2 ml each of ninhydrin and glacial acetic acid were added. The solution was boiled in water bath for 30 mins and then transferred into an ice bath. After 30 min, 4 ml toluene was added and the test tube was shaken vigorously. The upper red chromophore (toluene layer) was taken and absorbance was calculated at 520 nm. Toluene was used as blank reference.

2.5. Statistical analysis

Pearson's coefficient for correlation was statistically analyzed at a significance level of $P < 0.05$ and $P < 0.01$. The statistical significance of differences among means was determined by one-way analysis of variance (ANOVA).

III. RESULTS & DISCUSSION

3.1. Pb Uptake in *S. nigrum*

Pb uptake in roots and shoots of *S. nigrum* is shown in Tables 1. Pb content significantly increased with increasing metal concentration and the exposure period at all the testing days. A steady increase in Pb uptake and accumulation was observed for all treatments. Roots showed a progressive accumulation of Pb as a function of the external medium. Pb was preferentially accumulated in roots. Significantly, a maximum Pb uptake of 32.6 mg/kg (in root) and 6.1 mg/kg (in shoot) was observed at highest Pb-250 mg/kg treatment at 90 DAT. At all the sampling, maximum uptake of Pb was observed in highest dose. Accumulation of Pb in roots was found to be 5.3-10.6 times higher than that of shoots. Metal accumulation in root tissue can be accomplished either through deposition of the metal ions along the cell wall and/or inside the cell in the vacuoles. Pb was not observed in the control plants at any sampling stage. One Way Analysis of Variance (ANOVA) indicates a statistical significant difference ($P \leq 0.001$) in the mean metal content in shoots and roots of *S. nigrum* in response to Pb content. *S. nigrum* was found to tolerate Pb concentrations up to 250 mg/kg without showing any toxicity symptoms. This confirms the ability of this plant to establish and grow well in Pb contaminated soil.

Weed plants are generally considered to have great potential for use in phytoremediation due to their extensive fibrous root systems and relatively robust characteristics, thus helping establish a strong rhizosphere through contaminated soils (Qixing et al. 2011). Many phytoremediation studies have shown the potential of weed species to remove heavy metals from contaminated environment. Weeds such as *Poa annua* (for Cu, As), *Tephrosia purpurea* (for Mn), *Cannabis sativa* (for Cr), *Solanum nigrum* (for Mn), *Dissotis rotundifolia* and *Kyllinga erecta* (for Pb), *Calotropis procera* (for Zn, Mn, Cd, Cu), *Withania somnifera* (for Cu, Mn, As), *Eclipta alba* (for Cu, Mn, As), *Heliotropium ellipticum* (for Cu, Mn, As), *Cannabis sativa*, *Solanum nigrum* and *Rorippa globosa* (for Cd) showed good phytoremedial potential suggesting the use of these weedy plants for remediation of heavy metal-polluted soils (Varun et al. 2012; Girdhar et al. 2014; Lum et al. 2014).

The uptake of toxic metals, their translocations to plant parts and the degree of tolerance to them are dependent on metal speciation, and on the metabolism of the plants that may vary from plant to plant (Prasad 1999). Baker and Brooks (1989), Baker and Walker (1990), have all demonstrated selective heavy metal uptake in different species of *Thlaspi*. Most non-essential metals are likely excluded from plant uptake or are quickly immobilized in the plant (Fodor et al. 1998). This could be a possible reason for much higher uptake of Pb in the test plant. It has been shown that Pb accumulation in roots is significantly higher than the shoots, possibly because of the low Pb translocation from roots to shoots (Cunningham et al. 1995; Verma and Dubey, 2003) Metal accumulation in root tissue can be accomplished either through deposition of the metal ions along the cell wall and/or inside the cell in the vacuoles (Salt and Kramer 2000). The sequestration of specific metal ions or metal-chelate complexes in the root cells is highly dependent on the metal ion in question. Pb can be deposited or precipitated in the nucleus of root cells; it can also be found in the cytoplasm of root cells in association with precipitates which can be formed in membrane inclusions, vesicles or organelles (Castellino et al. 1995).

All the samples tested in the present investigation show greater accumulation of metal in the roots than the above ground shoot, this could be attributed to the increased metal adsorption on the root surface, being facilitated by relatively less mobility of metals in the root zone (Hasan et al. 2007). As plant roots are in direct contact with metals in contaminated soil and must act as the conduit for transfer of metal to the stem and leaves, their response to the high metal concentration is important. The differences in root and shoot uptake can possibly be explained by the fact that one of the normal function of root is to selectively acquire ions from the soil solution, whereas shoot tissue does not normally play this role. The results obtained in the present investigation are in conformity with the findings of Kadukova et al. (2004) and by other research groups (Lutts et al. 2004) which showed significant amount of metal accumulation in roots and shoots of plants as in this study, and together with the fact that there was no reduction in growth. The findings suggest that *S. nigrum* could be a possible candidate for Pb remediation.

Table 1: Pb uptake and accumulation (mg/kg) in *S. nigrum* (root/shoot) at successive days of study

Treatments (mg/kg)	25DAT		50DAT		75DAT	
	Root	Shoot	Root	Shoot	Root	Shoot
Pb-25	3.7±0.6	0.51±0.2	5.6±1.3	0.72±0.2	6.8±1.1	0.87±0.1
Pb-50	6.6±1.1	0.62±0.1	9.1±1.7	0.95±0.1	10.9±1.3	1.2±0.3
Pb-100	8.5±0.8	1.2±0.2	11.6±2.2	1.6±0.4	14.7±2.6	2.6±0.6
Pb-200	11.8±1.7	1.6±0.3	18.4±3.4	2.5±0.8	23.3±3.1	3.7±1.1
Pb-250	17.9±2.3	2.8±0.6	26.4±2.8	4.2±1.3	32.6±2.8	6.1±1.4

DAT: Days after treatment

3.2. Proline Content

Proline accumulation, accepted as an indicator of environmental stress, and is also considered to have important protective roles (Schat et al. 1997). The plants exposed to heavy metals seem to induce accumulation of free proline (Hayat et al. 2012). The data regarding proline accumulation in *S. nigrum* is presented in fig. 1. At all the testing days, all treatments showed higher levels of proline in comparison to the control. The value of proline accumulation increases with the increased in the concentration of Pb in soil. Proline content varied from 0.60 – 1.32 µg/g f.wt. (at 30DAT); 0.38 – 0.70 µg/g (at 60DAT) and 0.11 – 0.27 µg/g (at 90 DAT). Proline plays important roles in osmoregulation, protection of enzymes, stabilization of the machinery of protein synthesis, regulation of cytosolic acidity, and scavenging of free radicals. It has been proposed by many workers that proline accumulates in plants to counteract stress induced effects. In the present investigation also all treatments showed higher levels of proline accumulation as compared to control. Application of Pb in soil appeared to increase the proline values up to 26 times that of the control. Thus, the data in the present clearly indicates that increase in proline content is related to increase in metal stress. Similar findings were reported by other workers also in *Oryza sativa* (Roy et al. 1992), *sunflower* (Kastori et al. 1992) and *Brassica juncea* (Singh and Tiwari 2003).

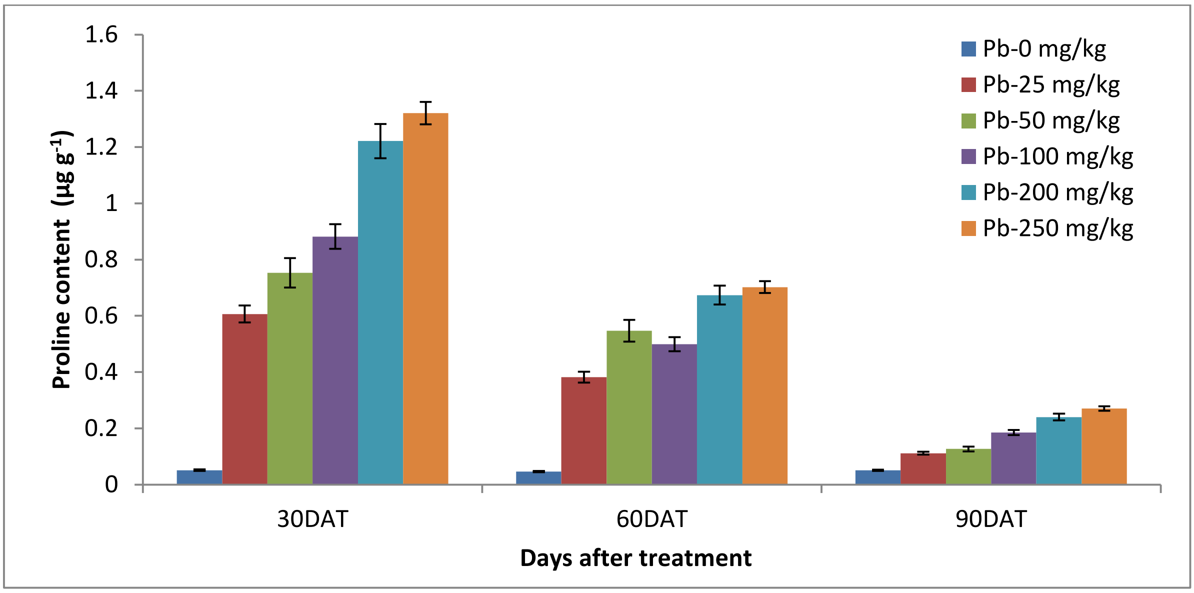

Fig. 1: Proline content (µg/g) in *S. nigrum* at successive days of study

3.3. Chlorophyll content

Metals like Cd, Pb, Zn, Cr, etc. when present in high concentration in soil show potential toxic effects on overall growth and metabolism of plants (Agrawal and Sharma 2006). The data regarding chlorophyll content in leaves of *S. nigrum* is presented in fig. 2. It is clearly evident from the figure that chlorophyll content of leaves was influenced by the Pb treatments applied. Application of Pb decreases chl 'a', chl 'b' as well as total chlorophyll content in leaves with the increasing metal concentrations up to 60 DAT in all the treatments. After 60 DAT chlorophyll content in all treatments stabilized and the values were nearly equal to control showing the adaptability of the plant to the Pb contamination. Chl 'a' varied from 0.98 – 1.89 mg g^{-1} f.wt.; chl 'b' varied from 1.04 – 1.9 mg g^{-1} f.wt.; and total chlorophyll varied from 2.02 – 3.82 mg g^{-1} f.wt. at 30 DAT to 1.75 – 1.92 mg g^{-1} f.wt., 1.71 – 1.86 mg g^{-1} f.wt., and 3.47

– 3.78 mg g^{-1} f.wt., respectively at 90 DAT. Decrease in the total chlorophyll content has been well documented under heavy metal stress and may reflect the level of photosynthetic activity in plants (Panda and Choudahry 2005; Jiang et al. 2007). Heavy metals are reported to inhibit chlorophyll biosynthesis, particularly by inhibiting 2 – aminolevulinic acid hydrogenase and protochlorophyllide reductase (Miyadate et al. 2011). Pb may interfere with different steps of Calvin cycle, resulting in the inhibition of photosynthetic substances and in poisoning of the cell cytoplasm (Pahlasson 1989). Similar results were reported by Phetsombat et al. 2006; Pandey et al. 2007.

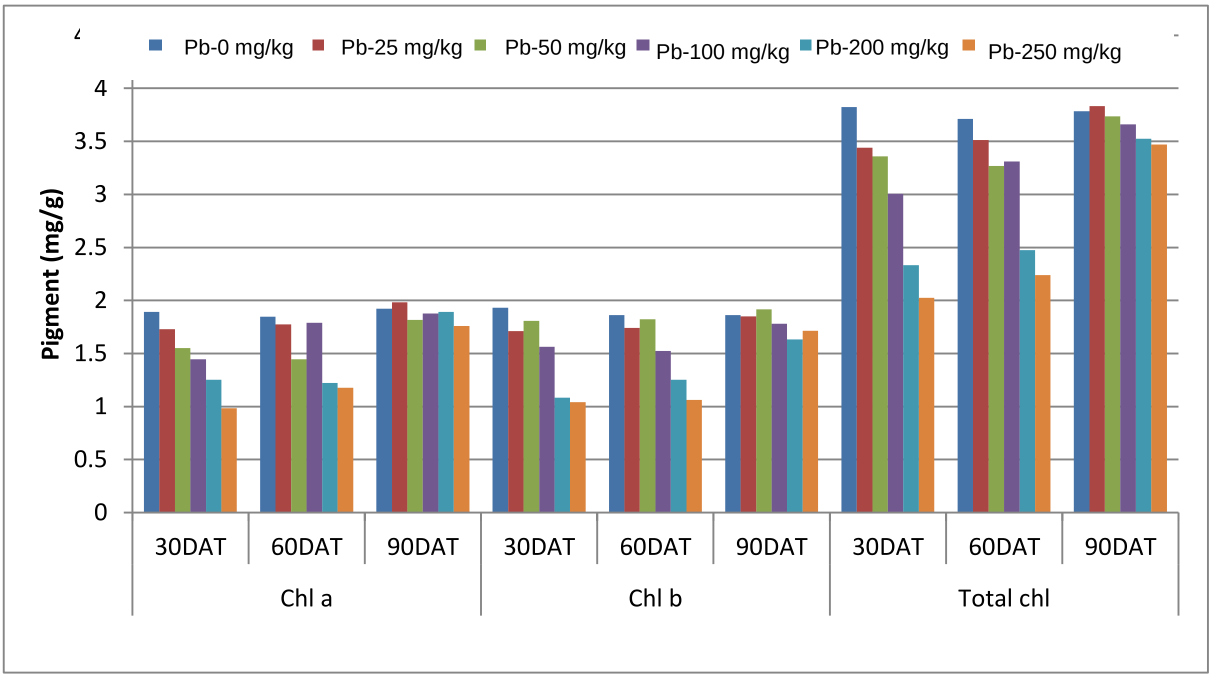

Fig.2: Chlorophyll pigments in *S. nigrum* at successive days of study. (DAT= Days after treatment)

3.4. Growth

Addition of Pb to soil inhibited growth of *S. nigrum* in terms of shoot and root length. The highest value for root and shoot length among the treatments was observed at low Pb concentrations only (Table 2). Lead has also been known to negatively affect root and stem elongation as well as leaf expansion in *Allium species* (Gruenhale and Jager 1985). The degree to which root elongation is inhibited depends upon the concentration of lead and ionic composition and pH of the medium (Goldbold and Hutterman 1986). Concentration-dependent inhibition of root growth has been observed in *Agrostis capillaries* seedlings (Symenoidis et al. 1985) and in *Sesamum indicum* (Kumar et al. 1992).

Table 2: Root length and Shoot length (in cm) of *S. nigrum* at successive days of study.

Treatments	Root length (cm)			Shoot length (cm)		
	30DAT	60DAT	90DAT	30DAT	60DAT	90DAT
Pb-0 mg/kg	18.6±2.3	24.1±3.1	31.6±4.2	66.1±3.3	92.3±4.6	108.6±6.2
Pb-25 mg/kg	17.4±1.8	21.5±2.2	29.2±1.7	62.3±2.6	82.6±3.2	100.8±2.3
Pb-50 mg/kg	10.5±1.4	13.9±1.9	21.2±0.8	37.4±1.8	53.4±1.9	73.1±1.1
Pb-100 mg/kg	9.4±0.8	13.6±2.3	22.7±2.3	33.5±3.6	53.1±0.6	78.3±3.4
Pb-200 mg/kg	8.5±1.2	11.5±1.4	18.5±2.1	30.4±1.5	44.0±2.8	63.8±0.6
Pb-250 mg/kg	7.2±0.7	9.9±1.8	16.7±2.7	25.3±2.7	37.9±3.1	57.5±3.1

It has also been reported that even at relatively low concentrations it alters plant metabolism (Van Assche and Clijsters 1990). Total biomass of plant was calculated and compared with their respective controls to assess the effect of Pb contamination on the overall growth of plant. All the treatments show less biomass than the control value at all sampling stages (fig. 3). Biomass decreases with the increase in Pb concentration in the growth matrix with maximum value obtained in control (Pb- 0 mg/kg) and the minimum value obtained in Pb-250 mg/kg treatment. Pb was shown to interfere with the increased tissue permeability, hence the increase in toxicity, the increased cross linking of pectins in the middle lamella of cell wall which might inhibit cell expansion (Poschenrieder et al. 1989), and the direct and indirect effect on the growth hormone, auxin metabolisms or auxin carriers.

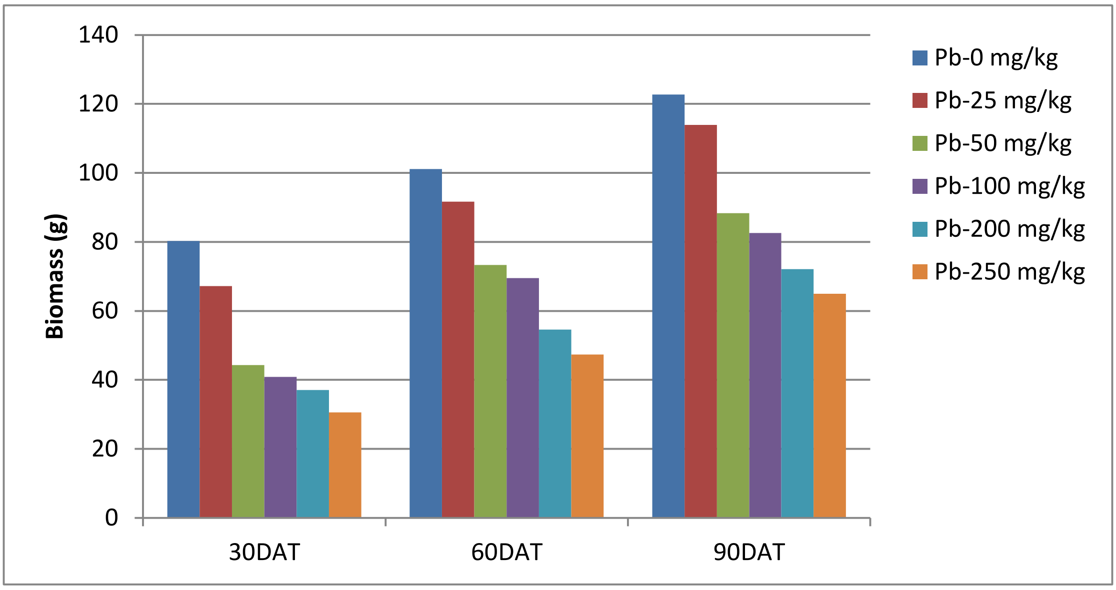

Fig. 3 Biomass (g) of *S. nigrum* at successive days of study.

IV. CONCLUSION

Solanum nigrum undertaken in the present study showed a potential for Pb bioaccumulation, and also maintained its growth and physiology. Given the good tolerance, fast growth, high accumulation, and global distribution, we propose that *S. nigrum* is a potential weed to remediate moderately Pb contaminated soil. However, it should still be tested in the field under metal-contaminated conditions.

V. REFERENCES

[1]. Agrawal, V., & Sharma, K. (2006). Phytotoxic effects of Cu, Zn, Cd and Pb on in vitro regeneration and concomitant protein changes in *Holarrhena antidysentrica*. *Biologia Plantarum*, 50, 307–310.

[2]. Arnon, D. I. (1949). Copper enzymes in isolated chloroplasts: Polyphenol oxidase in *Beta vulgaris*. *Plant Physiology*, 24, 1–15.

[3]. Baker, A. J. M., & Brooks, R. R. (1989). Terrestrial higher plants which hyperaccumulate metallic elements-a review of their distribution, ecology and phytochemistry. *Biorecovery*, 1, 81–126.

[4]. Baker, A. J. M., & Walker, P. I. (1990). Ecophysiology of metal uptake by tolerant plants. In A. J. Shaw (Ed.), *Heavy metal tolerance in plants evolutionary aspects* (pp. 155–178). CRC Press: Boca Raton, FL.

[5]. Baker, A.J.M., & Brooks, R.R. (1989). Terrestrial higher plants which hyperaccumulate metallic elements- a review of their distribution, ecology and phytochemistry. *Biorecovery*. 1:81–126.

[6]. Bates, L. S., Waldren, R. D., & Teare, T. D. (1973). Rapid determination of free proline for water stress studies. *Plant and soil*, 39, 205–207.

[7]. Castellino, N., Sannolo, N., & Castellino, P. (1995). Inorganic Lead Exposure: metabolism and Intoxications. Lewis publisher, CRC press, Boca raton, Fl

[8]. Chaney, R.L., Malik, M., Lil, Y.M., Brown, S.L., Brewer, E.P., Angle, J.S. & Baker, A.J.M. (1997). Phytoremediation of soil metals. *Current Opinion in Biotechnology*. 8:279–284.

[9]. D'Souza, R., Varun, M., Masih, J., & Paul, M. S. (2010). Identification of *Calotropis procera* L. as a potential phytoaccumulator of heavy metals from contaminated soils in Urban North Central India. *Journal of Hazardous Material*, 184, 457–464.

[10]. Fodor, F., Cseh, E., Varga, A., & Zaray, G. (1998). Lead uptake, distribution and remobilization in cucumber. *Journal of Plant Nutrition*, 21, 1363–1373.

[11]. Girdhar, M., Sharma, N. R., Rehman, H., Kumar, A., & Mohan, A. (2014). Comparative assessment for hyperaccumulatory and phytoremediation capability of three wild weeds. *3 Biotech* **4**(6): 579–589.

[12]. Goldbold, D. J., & Hutterman, A. (1986). The uptake and toxicity of mercury and lead to spruce (*Picea abies*) seedlings. *Water, Air, & Soil Pollution, 31*, 509–515.

[13]. Gruenhage, L., & Jager, H. J. (1985). Effect of heavy metals on growth and heavy metal content of *Allium porrum* and *Pisum sativum*. *Angew Botany*, 59, 11–28.

[14]. Hasan, S. H., Talat, M., & Rai, S. (2007). Sorption of cadmium and zinc from aqueous solutions by water hyacinth (*Eichhornia crassipes*). *Bioresource Technology*, 98, 918–928.

[15]. Hayat, S., Hayat, Q., Alyemeni, M. N., Wani, A. S., Pichtel, J., & Ahmad, A. (2012). Role of proline under changing environments. *Plant Signaling and Behavior*, 7(11), 1456–1466.

[16]. Jiang, H. M., Yang, J. C., & Zhang, J. F. (2007). Effects of external phosphorus on the cell ultrastructure and the

chlorophyll content of maize under cadmium and zinc stress. *Environmental pollution*, 147, 750–756.

[17]. Johnson, M.S., & Eaton, J.W. (1980). Environmental contamination through residual trace metal dispersal from a derelict lead-zinc mine. *Journal of Environmental Quality*, 9: 175–179.

[18]. Kadukova, J., Papadontonakis, N., Naxakis, G., & Kalogerakis, N. (2004). Lead accumulation by the salt-tolerant plant *Atriplex halimus*. In: C. Moutzouris, C. Christodoulatos, D. Dermatas, A. Koutsospyros, C. Skanavis, & A. Stamou (Eds.), *Proceedings of the International Conference on Protection and Restoration of the Environment VII* June 28–July 1, Mykonos, Greece.

[19]. Kastori, R., Petrovic, M., & Petrovic, N. (1992). Effect of excess lead, cadmium, copper and zinc on water relations in sunflower. *Journal of Plant Nutrition*, 15, 2427–2439.

[20]. Kumar, G., Singh, R. P., & Sushila. (1992). Nitrate assimilation and biomass production in *Sesamum indicum* L. seedlings in lead enriched environment. *Water, Air, & Soil Pollution,* 215,124–215.

[21]. Lee, S.Z., Chang, L., Yang, H.H., Chen, C.M., & Liu, M.C. (1998). Absorption characteristics of lead onto soils. *Journal of Hazardous Material*, 63, 37–49.

[22]. Lum, A. F., Ngwa, E. S., Chikoye, D., & Suh, C. E. (2014). Phytoremediation potential of weeds in heavy metal contaminated soils of the Bassa Industrial Zone of Douala, Cameroon. *International Journal of Phytoremediation,* 16(3), 302–19.

[23]. Lutts, S., Lefère, I., Delpéré, C., Kivits, S., Dechamps, C., Robledo, A., & Correal, E. (2004). Heavy Metal Accumulation by the Halophyte Species Mediterranean Saltbush. *Journal of Environmental Quality,* 33, 1271–1279.

[24]. Miretzky, P., Saralegui, A., & Fernandez, C. A. (2004). Aquatic macrophytes potential for the simultaneous removal of heavy metals (Buenos Aires, Argentina). *Chemosphere,* 57(8), 997–1005.

[25]. Miyadate, H., Adachi, S., Hiraizumi, A., Tezuka, K., Nakazawa, N., Kawamoto, T., Katou, K., Kodama, I., Sakurai, K., Takahashi, H., Satoh-Nagasawa, N., Watanabe, A., Fujimura, T., & Akagi, H. (2011). OsHMA3, a P18-type of ATPase affects root-to-shoot cadmium translocation in rice by mediating efflux into vacuoles. *New Phytologist,* 189, 190–199.

[26]. Pahlasson, A. M. B. (1989). Toxicity of heavy metals (Zn, Cd, Cu, Pb) to vascular plants. *Water Air and Soil Pollution,* 47, 278–319.

[27]. Paivoke, A.E.A. (2002). Soil lead alters phytase activity and mineral nutrient balance of *Pisum sativum*. *Environmental and Experimental Botany*, 48, 61–73.

[28]. Panda, S. K., & Choudhary, S. (2005). Chromium stress in plants. *Brazilian Journal of Plant Physiology,* 17(1), 19–102.

[29]. Pandey, S., Gupta, K., & Mukherjee, A. K. (2007). Impact of cadmium and lead on *Catharanthus roseus* – A phytoremediation study. *Journal of Environmental Biology,* 28, 655–662.

[30]. Phetsombat, S., Kruatrachue, M., Pokethitiyook, P., & Upatham, S. (2006). Toxicity and bioaccumulation of cadmium and lead in *Salvinia cucullata*. *Journal of Environmental Biology,* 27(4), 645–652.

[31]. Poschenrieder, C., Gunes, B., & Barcelo, J. (1989). Influence of cadmium on water relation, stomatal resistance, and abscisic acid content in expanding bean leaves. *Plant Physiol*ogy, 90, 1365–1371.

[32]. Prasad, M. N. V. (1999). Metallothioneins and metal binding complexes in plants. In M. N. V. Prasad, & J. Hagermeyer (Eds.), *Heavy metal stress in plants: from molecules to ecosystems* (pp. 51–72). Berlin: Springer verlag.

[33]. Qixing, Z., Zhang, C., Zhineng, Z., & Weitao, L. (2011). Ecological Remediation of Hydrocarbon. Contaminated Soils with Weed Plant. *Journal of Resources and Ecology,* 2(2), 97–105.

[34]. Roy, D., Bhumnia, A., Basu, N., & Banerjee, S. K. (1992). Effect of NaCl salinity on metabolism of proline in salt sensitive and salt-resistant cultivars of rice. *Biologia Plantarum,* 34, 159–162.

[35]. Salt, D. E. & Kramer, U. (2000). Mechanisms of metal hyperaccumulation in plants. In I. Raskin, & B. D. Ensley (Eds.), *Phytoremediation of toxic metals: using plants to clean-up the environment* (pp. 231–246). New York : John Wiley & Sons, Inc.

[36]. Salt, D. E., Prince, R. C., Pickering, I. J. & Raskin, I. (1995). Mechanisms of cadmium mobility and accumulation in Indian Mustard. *Plant Physiology,* 109, 1427–1433.

[37]. Schat, H., Sharma, S. S. & Vooijs, R. (1997). Heavy metal–induced accumulation of free proline in a metal–tolerant and a non-tolerant ecotype of *Silene vulgaris*. *Physiology of Plant,* 101(3), 477–482.

[38]. Singh, P. K., & Tewari, R. K. (2003). Cadmium toxicity induced changes in plant water relations and oxidative metabolism of *Brassica juncea* L. plants. *Journal of Environmental Biology,* 24, 107–112.

[39]. Symenoidis, S. L., McNeilly, J., & Bradshaw, A. D. (1985). Differential tolerance of *Agrostis capillaries* to cadmium, copper, lead, nickel and zinc. *New Phytologist,* 101, 309–316.

[40]. Uraguchi, S., Watanabe, I., Yoshitomi, A., Kiyono, M., & Kuno, K. (2006). Characteristics of cadmium accumulation and tolerance in novel Cd-accumulating crops, *Avena strigosa* and *Crotalaria juncea*. *Journal of Experimental Botany,* 57(12), 2955–2965.

[41]. Van Assche, F., & Clijesters, H. (1990). Effects of metals on enzyme activity in plants. *Plant Cell Environment,* 13, 195–206.

[42]. Varun, M., D'Souza, R., Kumar, D., & Paul, M. S. (2011). Bioassay asmonitoring system for lead phytoremediation through *Crinum asiaticum* L. *Environmental Monitoring and Assessment,* 178, 373–381.

[43]. Varun, M., D'Souza, R., Pratas, J., & Paul, M. S. (2012). Metal contamination of soils and plants associated with the glass industry in North Central India: Prospects of phytoremediation. *Environmental Science and Pollution Research,* 19(1), 269–281.

[44]. Verma, S., & Dubey, R.S. (2003) Lead toxicity induces lipid peroxidation and alters the activities of antioxidant enzymes in growing rice plants. *Plant Science*, 164, 645–655.

[45]. Yang, X., Feng, Y., Zhenli, H., & Stoffella, P.J. **(**2005). Molecular mechanisms of heavy metal hyperaccumulation and phytoremediation. *Journal of*

Trace Elements in Medicine and Biology. 18(4):339–353.

[46]. Yang, Y.Y., Jung, J.Y., Song, W.Y., Suh, H.S., & Lee, Y. (2000). Identification of rice varieties with high tolerance or sensitivity to lead and characterization of the mechanism of tolerance. *Plant Physiology*, 124, 1019–1026.