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NICKEL RESILIENCE BY PLANT-MICROBE ASSOCIATION: A SUSTAINABLE APPROACH FOR REMEDIATION OF PYROTECHNIC CONTAMINATED SOIL

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Abstract: Pyrotechnics are notorious source of heavy metal discharge to the environment. Nickel is one of the ubiquitous metal present in the pyrotechnic composition of firework industries. Therefore, nickel will enter easily to the organism by bio transfer mechanism. Percolation of nickel alters the reaction within the photosynthetic apparatus and oxygen evolving complex in plants. Phytoremediation of nickel is authoritative resilience of future and the limitation of photosynthetic system ought to be improved. Accordingly, phytoremediation requires enhancer for the prominent success to sustain and removal of nickel by plants from soil. Microfungal organisms are known bio-enhancer due to their metabolic profiles and their role in plant growth promotion. This study is aimed to determine the efficiency of nickel removal from fireworks contaminated soil of Sivakasi. Nickel uptake, accumulation and tolerance were investigated with seedlings of *Gossypium hirsutum* L. During nickel treatment, biomass and photosynthetic pigments got reduced with increasing concentration of nickel chloride and sugar, protein, anthocyanin, catalase and peroxidase contents were increased, the biomass, growth and biochemical characters will regain and the other biochemical parameter got reduced. After the addition of enhancer, plant develops mechanism such as accumulation of anthocyanin and enhanced activities of antioxidant enzymes to overcome the ill effects of the metal ion and *G. hirsutum* absorbed 39.05mg/L of nickel from the contaminated soil (42.03mg/L). Therefore, we claimed this could be a significant attempt as potential phytoremediation strategy for heavy metal removal. Our findings also support to improving economical niche by using commercial plant to brings metal free soil and safeguard our environment.

IndexTerms: Phytoremediation, Nickel, Pyrotechnic chemicals, *Gossypium hirsutum*, *Aspergillus* sp., Metal recovery.

1. Introduction

Naturally heavy metal occurs in the environment and found in all plants in minimum quantities. Besides, heavy metals are required for plant growth and development. Huge amount of nickel released into the environment during fireworks industry, they are strongly dispersed into the soil (Malaieswari *et al*., 2016). Nickel is one of the most abundantly distributed in nature as a component of soil minerals (Neiborer et al., 1992). At higher concentrations nickel affect the plant growth and metabolism and produce visible signs of toxicity. The plant grown in high nickel content soil showed the nutrient balance impairment and caused disorder function in cell membrane. Nickel is toxic to plant growth and becomes a problem in acidic soil (Temp, 1991). Nickel has been reported to damage cell organelles and plasma membrane, thereby inhibiting the plant growth (Pavlokin *et al*., 2016).

Drinking water normally holds nickel at less than 10µg/L concentration assuming a daily intake of 1.5 L of water and level of 5-10µg Ni/L, from this view, intake of nickel by adults is approximately range between 7.5 µg and 15.0 µg. But the uptake of large quantities of metals results in respiratory failure, birth defects, asthma, chronic bronchitis and heart disorders (Mehjbeen *et al*., 2003). Higher level of heavy metals intake caused nervous disorders in human beings and excessive exposure to nickel can lead to severe damage of lungs, kidneys, skin dermatitis and cancer (Campel *et al*., 2006).

The results of several researches confirmed that high concentration of nickel in plant lead to the inhibition of germination, competition with other essential metal ions, alteration of many enzymatic activities, disruption of cell structure and dehydration, wilting, oxidative stress etc., (Seregin *et al*., 2003), reduce the rate of shoot and root growth, biomass production, leaf spotting and foliar necrosis (Pandey *et al*., 2006), and chlorosis and red brownish colorization (Rathor *et al*., 2017).

Nickel accumulation in cell wall shows a significant part in nickel toxicity in plants and it tempts the inhibition of root growth by suppressing the cell division (Seregin *et al*., 2003). Photosynthesis is a energetic metabolic process for the development and productivity of plant, which is exaggerated by nickel. It impairs the and dislocate the structure of photosynthetic apparatus, obstruct the activities of enzymes involved in Calvin cycle and lack of CO_2 caused by stomatal closing (Seregin *et al.*, 2006). An excess nickel also affect nutrient absorption, photosynthesis inhibition by means of affecting oxygen evolving complex, damage the enzyme mechanism and the oxidation site of photosystem II, transpiration and transportation (Shaw *et al*., 2004).

Sivakasi is famous for fireworks factories and second largest producer in the world and capital of fireworks in India. For colourful effects of crackers, fireworks industries uses cocktail of pyrotechnic chemicals like various inorganic and heavy metals.

Remediation of heavy metals is inevitable to safeguard the environment from their noxious effects. Plant–microbe interactions have gained considerable attention due to the potential bioaccumulation of metals and metal uptake by microorganisms (Glick, 2010).

The focus of our study is to remediate the nickel from fireworks pyrotechnic contaminated soil. A time course pot experiment was conducted to detect the effect of nickel toxicity in plants and physiological parameter affected by fungal activity. *Gossypium hirsutum* L. was selected as the model plant for the remediation studies due to its ability to grow in nickel contaminated soil and withstand the drought condition. The indigenous fungus *Aspergillus* sp. treated with *G. hirsutum* L. prove to be effective in the removal of nickel from soil and hyper accumulate in plant vegetative parts. Finally the plants were burnt and the metals were recovered. Results obtained from this study encourage the increased effectiveness in phytoremediation enhanced by fungus.

2. Materials and methods

2.1. Experimental design

Gossypium hirsutum L. (Malvaceae) was selected for this study on the basis its bioaccumulation capacity, economic importance for fibres and non-food crop, so that the heavy metals do not enter intoa the food chain. The indigenous heavy metal reducing fungi were isolated from the contaminated site by serial dilution method. The fungus was preserved on agar slants. Efficient fungi *Aspergillus* sp. were screened and selected for further studies (Malaieswari *et al*., 2016).

The experimental pots were filled with 90% of their volume by mixture of red, black and sandy soil (1:1:1). The seedlings of *G. hirsutum* were planted in these pots. After ten days the seedlings were treated with different concentrations of nickel chloride ($NiCl_2$), viz. 2 mM, 4 mM, 6 mM, 8 mM and 10 mM (v/v) as five treatments along with control. The aqueous solutions of nickel were applied to the soil after the development of first leaf in the seedlings. The plants were watered with the respective concentrations of metals on every alternate day and continued for 30 days. Another set of plants treated with 8 mM nickel chloride (as 8 mM nickel treated plants was found to be optimum toxicity level by LSD analysis, Zar, 1984) was used for remediation studies. The 8mM concentration nickel treated plants were remediated with indigenous *Aspergillus* sp. for 30 days.

2.2. Physiological analysis

The plant growth parameters such as root length, shoot length, leaf area, fresh weight and dry weight were measured manually before and after the treatment.

2.3. Pigment analysis

Fresh leaves of control and experimental plants were deveined and cut into small bits. From the pooled leaf bits a sample of 100 mg was weighed. The leaf bits were homogenised in 100% acetone and supernatant was used for the estimation of photosynthetic pigments. The absorbance was measured at 662 nm, 645 nm,

and 470 nm for chlorophyll a, chlorophyll b and carotenoids respectively using spectrophotometer by Wellburn and Lichtenthalar methods (1984).

The leaf samples of experimental plants were incubated in 200 mL of extraction medium along with control. It consists of methanol, distilled water and HCl in the ratio of 50:20:1 respectively. The incubation was extended to 48 hrs at 4°C in dark condition with agitation. After 48 hrs the solution was collected and optical density was measured at 530 nm and 657 nm. The absorbance unit of anthocyanin content was estimated by Swain and Hill (1959) method.

2.4. Biochemical analysis

The biochemical factors such as total soluble sugar, protein, amino acid and proline content were subjected to analyse on 30th day after planting (DAP). Biochemical contents were estimated by the following methods: total soluble sugar was estimated using anthrone reagent by Jayaraman (1981) method, total soluble protein content was estimated by Lowry *et al*. (1951) method, free amino acids were estimated by ninhydrin assay (Jayaraman, 1981), and proline was quantitatively measured by Bates *et al*. (1973) method.

2.5. Enzymatic analysis

The enzymatic factors such as leaf nitrate content, *in vivo* nitrate reductase activity and, peroxidase and catalase activity assay were determined by using the method of (Cataldo *et al*. 1978), (Jaworski., 1971) and Kar and Mishra (1976) respectively.

2.6. Heavy metal analysis

Nickel concentration in control and *Aspergillus* sp. treated whole plants and vegetative parts of the plants were separately analyzed by atomic absorption spectrometry (AAS). Nickel quantity was determined by digesting dried plant sample in a mixture of nitric acid and perchloric acid (10:1 v/v) as described by (Ajaz Haja Mohideen *et al*. 2010).

2.7. Statistical analysis

All statistical analyses and variance tests were performed using two way ANOVA test. A 2 × 3 factorial analysis of variance (ANOVA) was used to evaluate the relationship between nickel treated and microbes treated plants. The morphometric parameters were determined with five independent replicates. Biochemical characters and enzymatic assays were carried out for five times. The statistical outputs from all ANOVAs are presented in Supplementary notes.

3. Results and discussion

3.1. Effect of nickel chloride on physiological characters of *G. hirsutum*

A probable mechanism for Ni uptake and accumulation in *G. hirsutum* by the accomplishment of *Aspergillus* sp. under Ni induced stress condensed the plant biomass and leaf colour. The significance observed between the nickels treated plants and remediated plants with control were described in supplementary note (Fig. S1, S2, S3, S4). The results of physiological assessment such as shoot length, root length, leaf area, fresh weight and dry weight decreased (fig.1) with the increasing concentration of nickel chloride. *G. hirsutum* plantlets grown in various concentration of nickel exhibited reduction in shoot length, root length, leaf area, fresh weight and dry weight with the shoot actually affected more than root length.

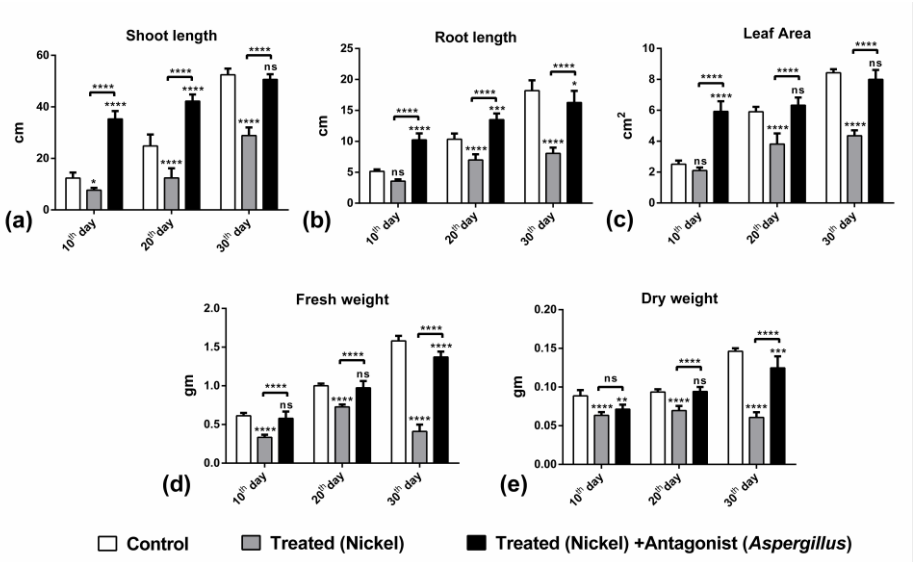

Fig. 1 Effect of nickel and *Aspergillus* sp. on the morphometric characters of *Gossypium hirsutum* L. Statistically significant difference between two groups, n=5, ($P < 0.005$).

3.1.1. Shoot length

The 8 mM nickel treated plants were used for the remediation studies with the indigenous fungi *Aspergillus* sp. After the remediation process the shoot length was improved from 4.12 cm to 7.96 cm. During the tenth day the nickel treated plant showed least significant result with the control and nickel with fungal treated plants shown extremely significant value with control plant. Likewise significant results were observed with the nickel treated and nickel with fungal treated plants. At 20th day, all the treated plants exhibited highly significant difference with control plants. On 30th day, the experiment has shown an extremely significant difference except the nickel with fungal treated plants (in significant difference) with control (Fig. 1 a)

3.1.2. Root length

After one month of treatment, the noteworthy descent was seen from 18.2 cm to 6.2 cm at 10 mM in root length of the treated plant. After remediation with fungus, the nickel treated plant roots were regained the length and weight. Extremely significant difference was observed between the fungal treated plants and control at 10th day interval. Highly significance values were observed at 20th day interval followed by least significant different value at the end of the experiment. Ni treated plants show extremely significant different value while compare to that of *Aspergillus* sp. treated plants (Fig. 1 b).

3.1.3. Leaf area

Leaf area was measured manually from the treated plants. The leaf area was declined from 8.44 cm^2 to 3.2 cm^2. The leaf area was retained after the treatment with *Aspergillus* sp. No significant values were observed between the remediated plants with control at all three intervals. Extremely significant differences were observed between the nickel treated and fungus treated plants (Fig. 1 c).

3.1.4. Fresh weight

The fresh weight of the nickel treated plants considerably decreased from 1.58 gm to 0.21 gm at 30thday of treatment. After the remediation process, the plants were regained the leaf weight. The remediated plant with control shows no significant different value at 10th day and 20th day interval. At 30th day, extremely significant differences were noticed with control. Similarly significant differences were observed from nickel treated plants with remediated plants (Fig. 1 d).

3.1.5. Dry weight

Dry weight of the plant biomass results shows an extremely significant value in all treated plants. After the 30th day of treatment, dry weight was also decreased from 0.146 gm to 0.034 gm. similar tendency was observed earlier by the researchers (Selvaraj *et al*., 2010).

During remediation studies, least significant differences were observed between the control and fungus treated plants at 10thday interval. No significant difference occurred between the nickels treated plants and remediated plants. At 20th day, no significant difference was observed between nickel treated plants with control and extremely significant difference was observed between nickel treated and *Aspergillus* sp. treated plants. At the end of the experiment also, an extremely different values were observed with nickel treated and remediated plants and higher significant difference noticed with the nickel treated plants with that of control (Fig. 1 e).

After the treatment with *Aspergillus* sp. shoot length, root length, leaf area, fresh weight and dry weight were increased. This indicates the *Aspergillus* sp. diminishes the effect of nickel chloride and enhances the growth of plant.

It has been stated in prior studies that Ni uptake and accumulation in plant condensed the plant growth and biomass by suppressing mineral uptake which disturbs the actual metabolic processes (Ali *et al*., 2011; Dheeba *et al*., 2015). The Ni induced stress is accountable for the generation of reactive oxygen species (ROS) which declined the growth and development of plants (Das *et al*., 2007; Singh *et al*., 2012). The addition of *Aspergillus* sp. significantly improved the growth and biomass of *G. hirsutum* indicating its promotive role in metal stress mitigation. The similar role of heavy metals uptake under metal stress was reported by several researchers (Najeeb *et al*., 2011; Zaheer *et al*., 2015).

3.2. Effect of nickel chloride on photosynthetic pigments

The effect of different concentrations of nickel chloride (2 mM to 10 mM) on photosynthetic pigments was illustrated in Fig. 2. Photosynthetic pigments were significantly decreased with increasing the level of nickel. After the 30th day, the reduction of chlorophyll a, chlorophyll b, anthocyanin and carotenoids were observed from 0.446 mg to 0.122 mg, 0.528 mg to 0.302 mg, 0.974 mg to 0.514 mg and 0.412 mg to 0.168 mg respectively at 10 mM nickel treatment when compared with the control.

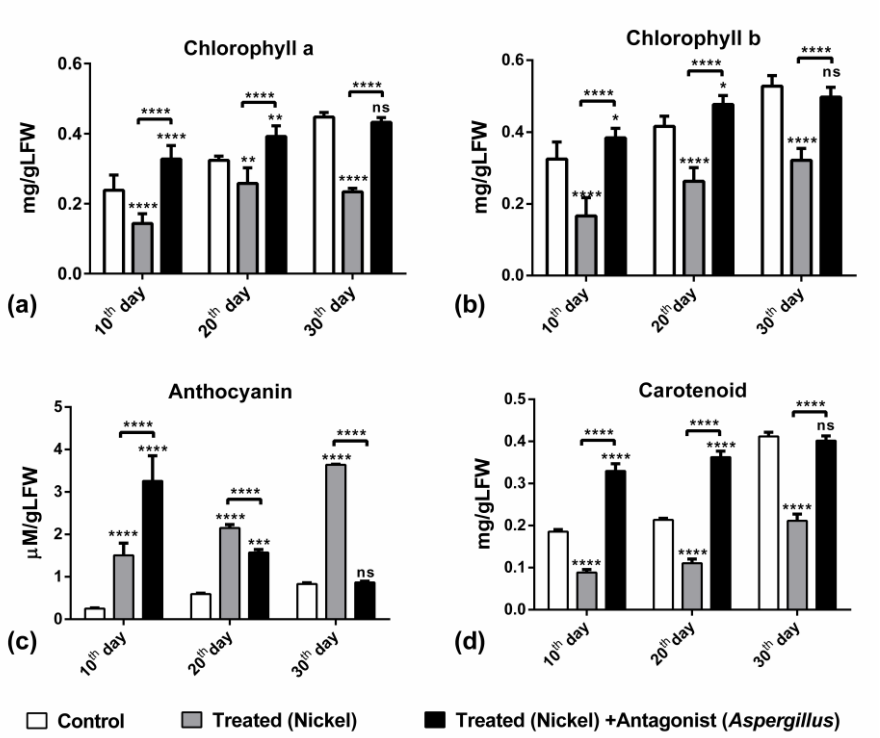

Fig. 2 Effect of nickel and *Aspergillus* sp. on the photosynthetic pigments of *Gossypium hirsutum* L. Statistically significant difference between two groups, n=5, ($P < 0.005$).

3.2.1. Chlorophyll a

The content of chlorophyll was decreased due to an increase in the concentrations of nickel chloride. After remediation with *Aspergillus* sp. extremely significant differences were observed between both nickel treated plants with control and nickel remediated plants at the interval of 10th day. At 20th day, least significant values were observed between the remediated plants with that of control and extremely significant values were obtained between the nickel treated and remediated plants. At the end of the experiment, remediated plants showed no significant results with the control and extreme differences with nickel treated plants (Fig. 2 a).

Bioaccumulation of nickel in *G. hirsutum* could result in the loss of pigment concentration significantly. Higher concentration of nickel can lead to chlorophyll degradation. Excessive nickel can induce oxidative stress in chloroplast which brings damage to the membranes by impacting the normal physiological functions of proteins, lipids and membrane (Fig.3).

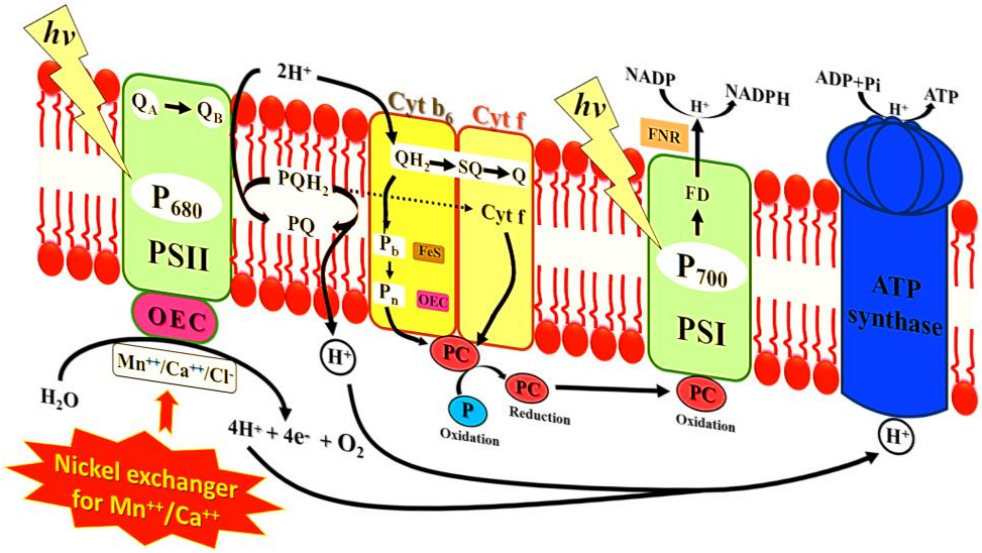

Fig. 3: Proposed effect of Ni exposure to plants at oxygen evolving complex in photosynthetic machinery. Alternation in organisation of oxygen evolving and light harvesting complexes. The nickel exchanges the manganese and calcium in the oxygen evolving complex. So nickel affect the OEC by block the release of hydrogen ions. Thus photosynthetic light reaction arrested by the heavy metal nickel. Heavy metal also affects the PSI, plastocyaninn and plastoquinine (Parmar *et al.*, 2013)

3.2.2. Chlorophyll b

After the remediation process, least significant difference and insignificant values were observed between the remediated plants with that of control at 10th, 20th and 30th day respectively. Extremely significant differences were observed between the remediated plants with nickel treated plants (Fig. 2 b).

3.2.3. Anthocyanin

Regarding anthocyanin content, there was marked increase from 0.862 mg to 3.932 mg at 10 mM after 30th day of treatment. The remediated plant shows extremely significant, higher significant and no significant difference with control at all the three intervals respectively. Extremely significant difference was observed between all remediated plants with nickel treated plants throughout the experiment (Fig. 2 c).

3.2.4. Carotenoids

All the remediated plants shows highly significant difference between the nickel treated plants and control plants except the 30th day treated plants which shows no significant difference (Fig. 2 d).

It has been reported that chlorophyll and carotenoid pigments gradually reduced in the tensioned leaves for their adaptation in cobalt chloride ($CoCl_2$) stressed condition (Gurusaravanan *et al*., 2012 and Marichali *et al*., 2016). The results of present study reveals increased Ni uptake ultimately reduced the Chlorophyll a, chlorophyll b and carotenoid contents in *G. hirsutum* (Fig. 2). It was confirmed that deformation of chloroplast ultra structure causes altered the shape and enlargement of thylakoids which is responsible for lowering the chlorophyll a, chlorophyll b and carotenoid contents in plant leaves (Parmar *et al*., 2013). This statement was corroborated with the findings of present study. The reduced plant growth might be the concern of low photosynthetic outcome due to the decreased chlorophyll and carotenoid contents. This observation evidently indicates photosynthetic process was brutally affected by increasing nickel concentration which impaired the photosynthesis and alter the structure of photosynthetic apparatus as already reported by (Seregin *et al*. 2001).

Accumulation of heavy metal inside the plant in higher concentrations induces the toxicity by modifying essential protein structure or replacing essential elements. It can be concluded from the reduction in biomass, production of chlorotic and necrotic leaves, loss of growth, browning of roots and disruption of photosynthetic process (Revathy *et al*., 2011).

The verdicts of our present study agreed with the conclusions of (Mahalakshmi *et al*., 2017) who stated a net decline in the capacities of photochemical efficiency of photosystem II and in the quantum yield of electron flow throughout PS II in the leaves of rye grass. Also reported the alterations in photosynthetic process and the absorption of essential nutrients lead to stunted plant growth.

The drop of chlorophyll was more than that of overall content due to associated with the modification of lower level of light harvest chlorophyll proteins (LHCPS) (Gill *et al*., 2012) and comparable changes was described by (Kushwaha *et al*. 2015) under zinc treatment. Likewise, reductions in the level of photosynthetic pigments including Chlorophyll a, Chlorophyll b and carotenoids, after exposure to heavy metals has been observed in many plant species (Singh *et al*., 2009; Singh *et al*., 2012; Kushwaha *et al*., 2015)

3.3. Effect of nickel chloride on biochemical characters

3.3.1. Total soluble sugar

Nickel decreased the total soluble sugar content in *G. hirsutum* leaves at gradually increasing with the nickel concentration. From our findings, the sugar content declined from 0.6 mg to 0.18 mg at 10 mM treatment was recorded. The reason behind the reduction of total soluble sugar in treated plants, due to the decrease of chlorophyll pigments by which photosynthetic process also decreased. So, the photosynthetic outcome of sugar production was arrested.

After the remediation, extremely significant differences were observed between the *Aspergillus* sp. treated and nickel treated plants. This finding clearly indicated that the *Aspergillus* sp. treated plant increases the sugar content than the nickel treated plants. By compared with the control extremely significant and insignificant differences were observed during the experiment (Fig. 4 a). The results of this study coincide with the findings of (Marichamy *et al*. 2015) in their work with barium.

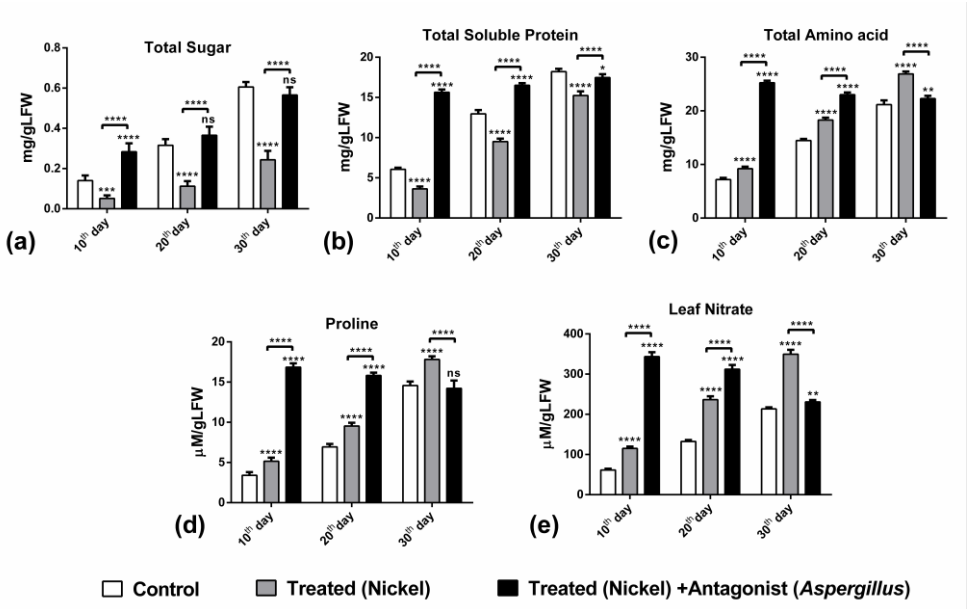

Fig. 4 Effect of nickel and *Aspergillus* sp. on the biochemical characteristics of *Gossypium hirsutum* L. Statistically significant difference between two groups, n=5, ($P < 0.005$).

3.3.2. Total soluble protein

From the results, it has been observed that the protein content got reduced from 18.2 µM to 14.52 µM by nickel treatment at 10 mM. Extremely significant differences were observed between the remediated plants with nickel treated plants and control throughout the course of the experiment (Fig. 4 b).

Plant protein denaturation will occur when they treated with higher metal concentration. The nickel inhibits the protein and amino acid formation by binding with the sulfhydryl group of protein and causing the abnormal structure of protein. At higher concentrations of nickel, the level of protein reduction is due to the blockage of protein synthesis by denaturing the responsible enzyme. Our results coincide with the results of (Seregin *et al*. 2006), who found that amino acid and protein content were high at the lower concentrations of zinc (10 mg/L and 25 mg/L). Heavy metals like arsenic and chromium also cause reduction of protein content in black gram and green gram seedlings (Bhupendra *et al*., 2014).

3.3.3. Free amino acids

The outcome of our research indicates free amino acids content in *G. hirsutum* significantly increase with increasing concentration of nickel. Extremely significant differences were observed between the remediated plants with nickel treated plants throughout the experiment. By comparing with the control, extremely significant differences was observed at the 10^{th} day and 20^{th} day in *Aspergillus* sp. treated plants and least significant difference was obtained from 30^{th} day experiment plant (Fig. 4 c). The increase in amino acid content indicates the disintegration of protein and blockage of protein synthesis due to heavy metal treatment.

3.3.4. Proline

The proline content was increased from 14.6 µM to 17.9 µM at 8mM treatment. After the treatment with *Aspergillus* sp. all remediated plants showed significant difference between nickel treated plants and control plants except the 30^{th} day treated plant, which exhibit no significant difference value (Fig. 4 d).

3.3.5. Leaf nitrate

The leaf nitrate content also emphatically increases with the increasing in the concentration of nickel. Higher concentration of nickel caused the higher level of accumulation of leaf nitrate content than that of the control. The 8mM nickel concentration increase the leaf nitrate content from 212 µM to 336 µM.

Our results exhibited extremely significant differences for all treated plants except 2mM, they exhibited no significant differences and least significant differences while compare with control, after remediation (Fig. 4 e). It was also clearly showed that the protein degradation occurs during nickel treatment, so that the availability of free amino acid content is high and the protein level is low.

It may be due to the degradation of protein or due to the biosynthesis of amino acid from the nitrate sources which were unutilized in the protein synthesis (Rastgoo *et al*., 2014). Our research findings were confirmed by the studies of (Schat., 1997) who found that high amount of proline was determined in black gram seedlings by increasing the concentration of lead. In root system, high content of proline remove hydroxyl radicals, sustain osmoregulation, check enzyme demolition and shrinkage the toxicity of heavy metals (Alia and Saradhi, 1991).

The same condition of increasing leaf nitrate with increased in the concentration of cadmium was observed in *Vigna radiata* by (Jayakumar and Ramasubramanian 2009) and results of this research work are highly similar to that of our study.

3.4. Effect of nickel chloride on antioxidant enzyme activities

The *in vivo* nitrate reductase activity was suppressed by the 10 mM nickel solution exposure and the activity was inhibited from 220 µM to 59 µM. Regarding catalase and peroxidase activity drastic upswing was attained with an increase in the nickel concentration from 0.88 µM to 2.72 µM and 24.6 µM to 38.6 µM respectively. Catalase and peroxidase activity was expressively increased in all the test plants than the control plants.

3.4.1. Nitrate reductase activity

The result of nitrate reductase activity expressed an extremely significant difference in all treated plants at 30th day interval. After the remediation process, the content of nitrate reductase enzyme declined from 36.7 µM to 32.4 µM, 28.2 µM and 26.02 µM respectively at the 10th day, 20th day and 30th day treatments. A relationship between treated and control plants exhibit least significant value. Higher level of significance was observed from *Aspergillus* sp. treated plants when compare with control. Extremely significant difference was observed between all the remediated plants with nickel treated plant. By comparing with that of remediated and control plants, the later showed higher significant, extremely significant and no significant difference at 10th day, 20th and 30th day respectively (Fig. 5 a).

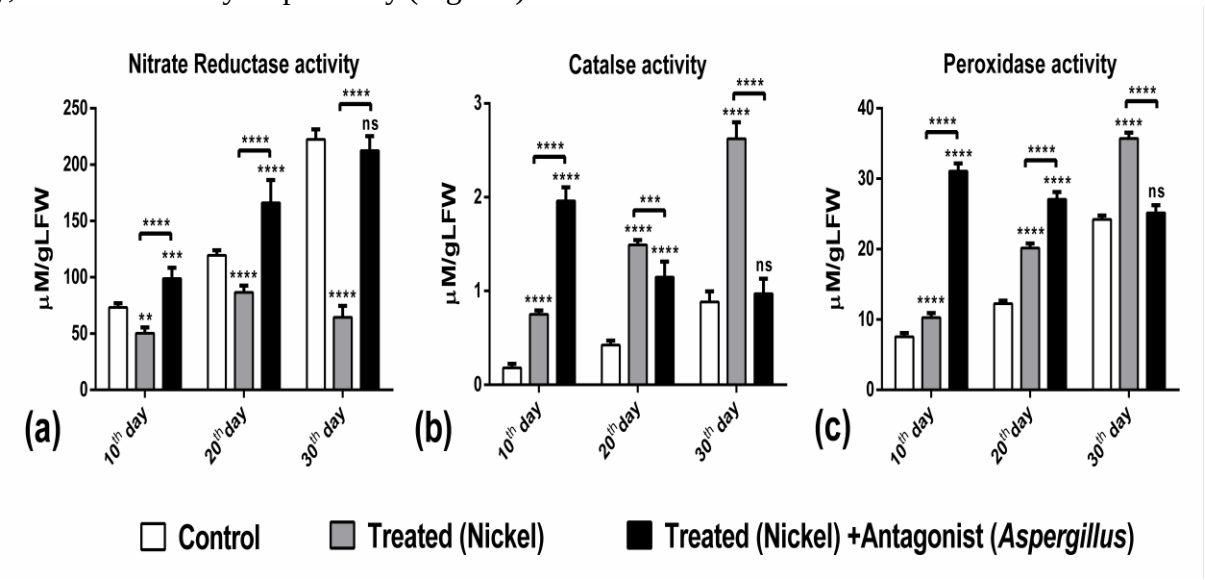

Fig. 5 Effect of nickel and *Aspergillus* sp. on the enzymatic activities of *Gossypium hirsutum* L. Statistically significant difference between two groups, n=5, ($P < 0.005$).

The leaf nitrate content was increased when the application of nickel as a result, nitrate reductase activity was decreased. Reduction in nitrate reductase activity with increased concentration of heavy metal like cadmium on *Vigna radiata* has been reported earlier (Jayakumar and Ramasubramanian, 2009).

3.4.2. Catalase activity

Catalase is an antioxidant and scavenging enzyme which are found to be increasing with the increase in the concentration of nickel chloride. All the remediated plants showed extremely significant differences between the nickel treated plants and control except the 20th day and 30th day nickel treated plant which shows highly significant differences and in significantly different values respectively (Fig. 5 b).

3.4.3. Peroxidase activity

Many research findings suggested that the peroxidase activity increases with the increase in the concentration of the nickel chloride.

After the remediation, all the remediated plants showed extremely significant difference between the nickel treated plants and control plants except that of 30th day nickel a treated plant that shows no significant difference value (Fig. 5 c).

Catalase is special type of enzyme which catalyses the degradation of H_2O_2, which is a natural metabolite and also toxic to plants (Balasimha, 1982). These findings strongly prove that under the heavy metal stress, catalase and peroxidase enzymes show significantly higher activities.

Plant cells are well fortified with a defense system together with antioxidant enzymes like catalase and peroxidase which can level the free radicals (Cho and Park, 2000). (Malar *et al*., 2014) also suggested that these antioxidant enzyme activities were increased under high concentration of heavy metal treatment.

Bioremediation studies with the *Aspergillus* sp. showed that the improvement in the morphometric characters after treatment. The level of chlorophyll, arytenoids, total soluble sugar and protein has increased after the application of *Aspergillus* sp. (Fig. 2 & 4). In contrary the anthocyanin, leaf nitrate, free amino acid, proline and the activity of enzymes such as catalase and peroxidase were start reduced after the application of *Aspergillus* sp. in plants pretreated with 8mM concentration of nickel chloride (Fig. 4 & 5).

3.5. Accumulation and recovery of nickel

AAS studies also confirmed the accumulation of nickel in *G. hirsutum* was increased with the increasing application of nickel chloride. About 20.68 mg/L nickel content was detected at 8 mM nickel chloride treatment. Due to the higher toxicity level, 8 mM nickel chloride treated plants were subjected for the remediation studies. After the introduction of *Aspergillus* sp. into the soil, the nickel toxicity level was decreased due to the microbial enhancement of nickel biotransformation.

After the *Aspergillus* sp. treatment, the nickel content in the plants was reduced. Microbes have various metabolic profiles which are absorbed the heavy metals and various chemicals for their metabolic processes. Thus the nickel level in the plants was reduced by the action of *Aspergillus* sp. Thus after the remediation 13.18 mg/L nickel only accumulate in the plants. The same phenomenon were also found in accordance with the research findings of (Sevugaperumal *et al*. 2012) by removing aluminium metal from fireworks contaminated site using *Padina* sp. as biosorbant.

Initially fireworks contaminated soil subjected for the present study contains 42.06 mg/L of nickel. G. *hirsutum* well grown from contaminated site, using chemicals as mineral supplement. The nickel extracted from soil and translocated into various parts of the plant. At the 10^{th} day, 8.24 mg/L nickel content was measured in the plants by AAS. After ten days the nickel content in plants increased to 15.02 mg/L. Finally 32.36 mg/L of nickel was removed from the contaminated soil by the absorbtion of plants.

The other set of experiment was performed by *G. hirsutum* plants with *Aspergillus* sp. After the introduction of *Aspergillus* sp. into the soil, the ability of metal absorbtion capacity was increased. After the 10^{th} day, the plant root absorbed 10.16 mg/L of nickel from soil. During second interval 18.94 mg/L of nickel accumulation was measured from the plant. At the end of the experiment, 39.05 mg/L of nickel was removed from soil by the enhanced activity of *Aspergillus* sp.

Higher concentration of nickel 13.32 mg/L was observed in the root of fireworks contaminated soil treated plants (Fig. 6). After the treatment with *Aspergillus* sp. the metal uptake capacity of the plant was increased to 16.58 mg/L from the soil. Compared to other parts of the plant, root cells were accumulated more amount of heavy metals than the shoot, leaves and cotton ball skin (Fig. 6).

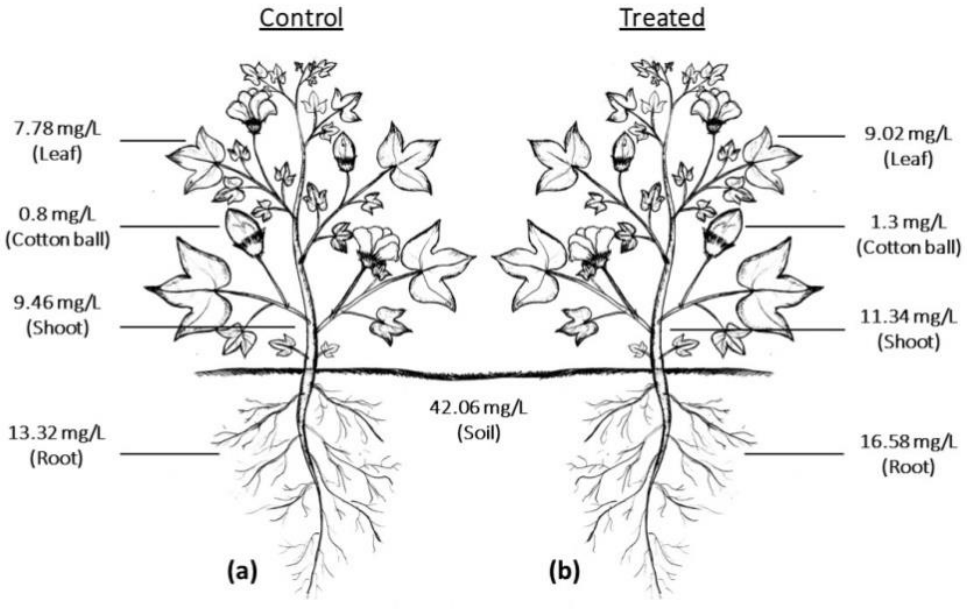

Fig. 6 Natural (a) and fungal assisted (b) phytoremediation by hyperaccumulator plant, *Gossypium hirsutum* L.

Leaves accumulated 9.46 mg/L of nickel in the control plants; it was increased to 11.34 mg/L after the treatment. Likewise the heavy metal in the shoot region was also increased from 7.78 mg/L to 9.02 mg/L after the treatment. After the treatment with *Aspergillus* sp., the uptake capacity of nickel increased from 0.8 mg/L to 1.3 mg/L in the cotton ball skin also.

Remarkably there is no heavy metal accumulation found in the flowers, cotton fibres and seed. Thus the heavy metals do not enter into the food chain. Our research finding shows that heavy metal only accumulated in the vegetative parts of the plant. They did not translocate and accumulate in the reproductive parts (Fig. 6). So our research outcome proves the economically value product from the plant like cotton and cotton seeds are free from heavy metals. After the treatment, the plants were taken out from the field, and were burned.

Plants naturally manage the contaminants by altering the nature of soil, which safeguards soil efficacy and fertility (Mench *et al*., 2009). After growing, the hyper-accumulating plants on a contaminated site, the biomass can be treated to recover the contaminant. The soil near plant roots (i.e., the rhizosphere) is an important habitat and ecosystem for microorganisms, including bacteria, fungi, algae, and protozoa. These microorganisms are naturally associated with plants in various ways (Azevedo *et al*., 2000; Hao *et al*., 2012). Microorganisms which are highly beneficial and play an important role in providing nutrients and can reduce the harmful effects of metals on plants. Some of these rhizosphere microorganisms can work directly on organic and inorganic pollutants using their own degradation capabilities, for instance, volatilization, transformation and rhizodegradation (Kuiper *et al*. 2004; Gadd, 2010).

4. Conclusion

The marked and severe effects of heavy metals have received the attention to convey solutions for the removal of the heavy metals from the Sivakasi fireworks contaminated area. Phytoremediation is a safe and pioneering method for remediating toxic metals. Phytoremediation with fungi is an emergent technology that seems to crack heavy metals problems without any lethal effects.

The results of this study revealed that *Aspergillus* sp. enhanced the efficiency of phytoremediation using *G. hirsutum.* The *Aspergillus* sp. transforms the nickel into non-toxic forms, which consequently facilitates phytoremediation. The accumulation of nickel was found to be reduced after the treatment of indigenous fungi *Aspergillus* sp. with *G. hirsutum*. The results clearly indicated that the fungi *Aspergillus* sp. proved that the potential remover of the nickel toxicity and the nickel only hyper accumulated in the vegetative parts of the plant *G. hirsutum* and not to translocate the reproductive parts. So, that the heavy metal does not enter the food chain in our ecosystem. *Aspergillus* sp. induced the plant to grow healthy without physiological changes. If *Aspergillus* sp. treated *G. hirsutum* plant introduced into the heavy metal contaminated area, surely the metal will be removed from the soil. Every year the heavy metal in the soil increased due to the increase in the rate of cracker manufacture. This will be the universal treatment to remove the toxic substance from such sites. Our research findings certainly improve the quality of life by increasing the consumption of metal free water, expose the living beings to metal free environment and reduce the skin related disease caused by the contaminants. This clearly holds a promising economical and eco-friendly metal bioremediation technology to develop a pollution free environment.

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