UNVERSITY OF DELHI

INNOVATION PROJECTS 2015-16

FINAL REPORT

1. PROJECT CODE: MH-311

2. PROJECT TITLE: Phytoremediation of Heavy Metals Contamination and Extraction of Nanoparticles.

3. NAME OF COLLEGE/INSTITUTION: Miranda House, University of Delhi.

4. PRINCIPAL INVESTIGATORS (NAME, DEPARTMENT, EMAIL, PHONE NO.)

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Ms. Anita Kumari, Assistant Professor, Department of Chemistry

Dr. Somdutta Sinha Roy, Assistant Professor, Department of Botany

5. MENTOR
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This is to certify that the research work carried out and the final report submitted bythe Project Investigators and the students of Innovation Project having Project code **MH 311**andtitle **"Phytoremediation of Heavy Metals Contamination and Extraction of Nanoparticles"**of **Miranda House**, University of Delhi, is original. Any plagiarism/academicdishonestyreported at any stage willbe ourresponsibility.

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Utilization Certificate

Innovation Project 2015-16

Project Code MH-311

Project Title: **Phytoremediation of Heavy Metal Contamination & Extraction of Nanoparticles**

Audited Financial Statement under Innovation Project scheme

College: Miranda House

Project Investigators: Dr. Sushma Moitra, Dr. Anita Kumari & Dr. Somdutta Sinha Roy

Grant sanctioned **Rs. 5, 00, 000/-** (Five lakhs only)				
S. No.	Budget Head	Amount Sanctioned	Total Amount Utilized	Amount Remaining
1.	Equipment/Consumable	2,25,000/-	2,25,000/-	Nil
2.	Travel	55,000/-	55,000/-	Nil
3.	Stipend	1,20,000/-	1,20,000/-	Nil
4.	Honorarium	25,000/-	23,374/-	1626/-
5.	Stationery	20,000/-	20,000/-	Nil
6.	Contingency	55,000/-	55,000/-	Nil
Total Amount Utilized		**Rs. 4, 98, 374/-** Four Lakh Nine Eight Thousand Three Hundred Seventy Four Only		
Amount Remaining		**Rs. 1626/-** One Thousand Six Hundred Twenty Six Only		

Certified that out of Rs. **5, 00,000/-** (Five Lakhs Only)
Sanctioned to Innovation Project Code MH-311 **Rs. 4, 98, 374/-** has been utilized
During the period of the project. The remaining amount **Rs. 1626/-** (One Thousand Six Hundred Twenty Six Only) is being returned back to the University.

Signature of project Investigators

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for A. Kumari
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Miranda House

Financial Audit Clearance
And Stamp of Charted Accountant

Dt. 04-11-2016

Final Report

1. Project Title: **Phytoremediation of Heavy Metals Contamination and Extraction of Nanoparticles.**
2. Project Code: **MH 311**
3. Abstract
4. Introduction

The biosphere contamination with heavy metals has increased sharply (Nriagu, 1979) posing major human health and environmental problems worldwide (Ensley, 2000). Heavy metals pose a grave problem as they do not degrade, and are persistent in the environment. In both developed and developing countries, a variety of biological resources have been used widely for cleanup of the metal polluted sites. Some of these technologies have become quite popular in the last one decade and presently are in the process of commercialization (Alcantara*et al*. 2000;Raskin and Ensley, 2000; Prasad, 2004). Phytoremediation refers to a diverse collection of plant-based technologies that use either naturally occurring or genetically engineered plants for cleaning contaminated environments (Flathman and Lanza, 1998).Studies have suggested that local native plant species are more appropriate for phytoremediation as they are already adapted to local conditions and also to avoid introduction of exotic species which can create ecological havoc.

Soil and water pollution occurs when untreated effluents enter the surface drains. Major heavy metal pollution occurs from dyes, unsorted disposal of

batteries with household garbage.Heavy metals identified in the polluted environment include Cu, As,Pb, Cd, Cr, Ni, Zn and Hg. Uptake of metals by plants in large amounts may produce toxicity in humans, and cause acute and chronic diseases. Excessive amounts of heavy metals in soil can negatively affect crop growth even leading to death of plants (Schmidt, 2003; Schwartz et al., 2003) and may also cause changes in the composition of soil microbial community, adversely affecting soil characteristics (Kurek and Bollag, 2004).

Thus, aim of the present project is to screen possible native plants both aquatic and terrestrial to come up with best candidates for cleaning up of specific heavy metal species and then extraction and characterization of nanoparticles from the plants.

5. Research problem/hypothesis/objectives

6. Methodology Techniques/Sampling /Tools/Materials

(A) Plant growth with heavy metal solutions (Short period growth):

1) Seeds of *Vigna radiata* (Mung bean) and *Sorghum bicolor* (Jowar)were washed under running tap water for about 10 minutes followed by a wash with detergent 3-4 times. They were then were transferred into a clean beaker and treated with 0.8% mercuric chloride solution for 5 minutes. The seeds were finally washed with distilled water thrice and inoculated on agar media containing heavy metal in the concentration of 10, 50 and 100 ppm.
2) After 48 h in the agar medium, % germination were noted down. Only germinated seeds were transferred to pots containing autoclaved vermiculite (15 seeds/ pot; 5 pots for each metal concentration)
3) Plants were irrigated with respective metal solution every alternate day (40 mL/ pot), length of the shoot and leaf was also measured. On the 10^{th} day, plants were taken out of their pots. Readings were taken for shoot length, root length, wet weight and dry weight (after oven drying) were noted.

4) Total of two sets for each plant were set up; results reflect the average of two sets.
5) Dried plant material is acid digested and prepared for analysis on Atomic absorption spectrophotometer for metal concentration.

(B) Growing the plants to study the abnormal cellular behaviours:

1) *Sorghum* and *Vigna* seeds were grown in metal solution with their root dipped in the solution for 48 hrs. The root tips were harvested and fixed into fixative containing absolute alcohol and acetic acid.
2) Slides were prepared for studying any kind of anomaly in mitosis by keeping the root tips in Cornea fluid for 2 minutes, the roots were squashed and drop of Acetocarmin was added. The slide was heated slightly and cover slip was lowered down .Slide was observed under compound microscope.

(C) MUFFLE FURNACE TECHNIQUE

Dry ashing procedure use a high temperature muffle furnace which is capable of maintaining temperature between 500 -600 Celsius . Water and other volatile materials are vaporized and organic substances are burned in the presence of the oxygen in air to carbondioxide , water and nitrogen .

1) The dried plant samples were converted into ash using the muffle furnace
2) Dried plant samples were weighed and transferred to crucible.
3) At one time 12 crucible containing the dried sample were kept inside the muffle furnace (which is set at 600 celsius) overnight.
4) These samples were removed from the muffle furnace next morning and ash was weighed and transferred to plastic vials for further analysis.

(D) SOLUTION PREPARATION FOR AAS

1) The entire ash sample is transferred to a clean and dry vials. Aqua regia was chosen as the most appropriate solvent for dissolving the ash.
2) 240 mL of aqua regia was prepared by mixing concentrating nitric acid and concentrated sulfuric acid in 1:3 ratio .

3) 3 ml of solvent is transferred to each vail containing ash sample . The prepared solution were diluted to 15 ml by adding 12 ml water to each vail.
4) Each solution was filtered through 0.2 micrometer filter paper using 5mL syringe and poured into labelled vials.
5) These samples were then sent for AAS determination.

7. Result and Discussion (main text, tables with titles, graphs and figures with legends) In detail

Both the experimental material studied, namely *Sorghum bicolor* and *Vigna radiata*, did not show any significant changes in their growth parameters when grown with 10, 50 and 100 ppm of the metals under study (Ni, Cr, Cd and Pb). Growth parameters considered includes root and shoot length, root and shoot wet weight and dry weight as well as percentage germination.

Amount of metal accumulation tested via Atomic Absorption Spectrophotometer for namely Cr, Pb and Cd in the root and shoot of *Vigna* and *Sorghum*, showed more accumulation in the roots as compared to shoots.Though, we have not been able to test the seeds, but it shows promise.

Presence of more than one nuclei in the root tip cells of both the plants.

8. Innovations shown by the project

Most of the phytoremediation studies only focus on the amount of heavy metal accumulated in the plant tissues. As an end point of the phytoremediation, the plant material is generally incinerated to reduce the total biomass. In the present study, we are working to make the bio-accumulated heavy metal to be recycled and reused by extracting them in the form of nanoparticles.

This unique approach, where the contaminated heavy metal can be reused and recycled using a green technology is the innovation of this study. Also, this technology gives a green way of manufacturing nanoparticles themselves.

9. Conclusion and Future direction

With increasing urbanization, land for agriculture and maintenance of biodiversity, is shrinking. Urbanization also meant increase in pollution levels and especially with heavy metals, making the land unfit for agriculture and human settlements. Heavy metals are known to get bioaccumulated in terrestrial as well as aquatic ecosystems, adversely effecting the plants, animals as well as humans.

The present study has not only taken up the challenge of phytoremediation of heavy metal contamination, but proposes to exploit this bioaccumulation in plants to extract out nanoparticles from plant residues.

Heavy metal pollution is a major challenge in today's world. It is a well studied problem and affects the human health in a wide range of ways. Whether it was Caesium 137 leakage from Chernobyl or lead accumulation in the soil around lead acid battery plants, heavy metals have effected the human health. Using phytoremediation for cleaning up heavy metal pollution has been well documented and a cheaper method.

This study opens up a new avenue and especially for those working in the field of ecological rehabilitation of soil and water. We have used common crop plants like *Vigna* and *Sorghum* and looked at the phytoremediation. Additionally, we are working on the phytoextraction of the heavy metals in the form of nanoparticles for commercial application.

10. References in APA format

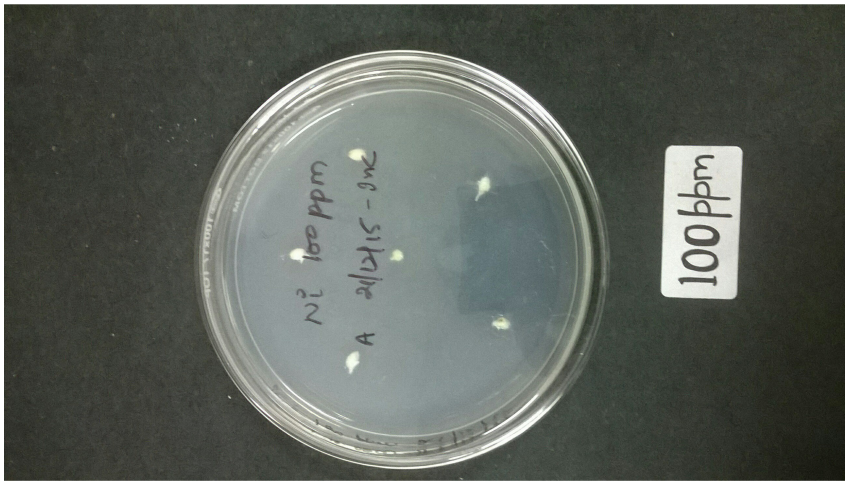
11. Publication/s from thework. (attach copies)

12. Conference Presentation/s (attach copies)

13. Patent/s and Technology Transfer (attach copies)

14. Media Coverage (attach copies)

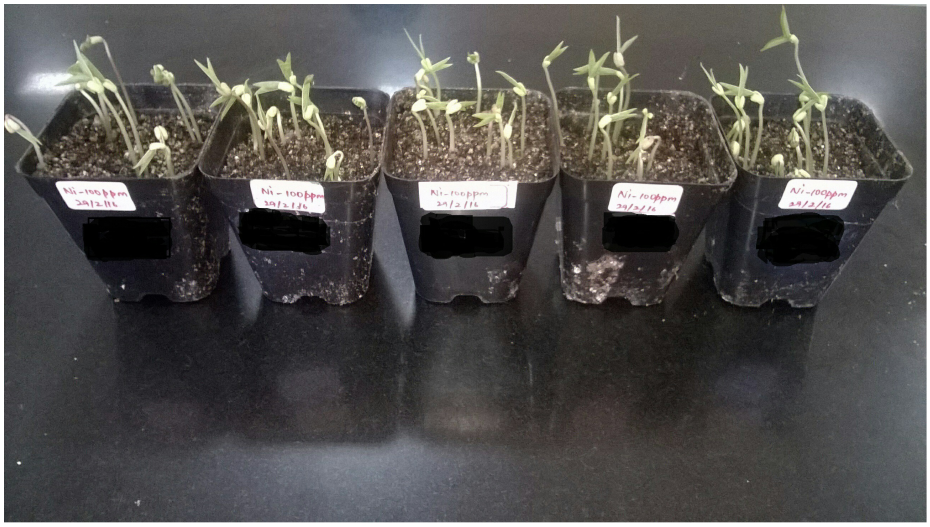
15. Pictures related to the project.



Seeds germinated in agar medium having 100ppm Nickel ion concentration.

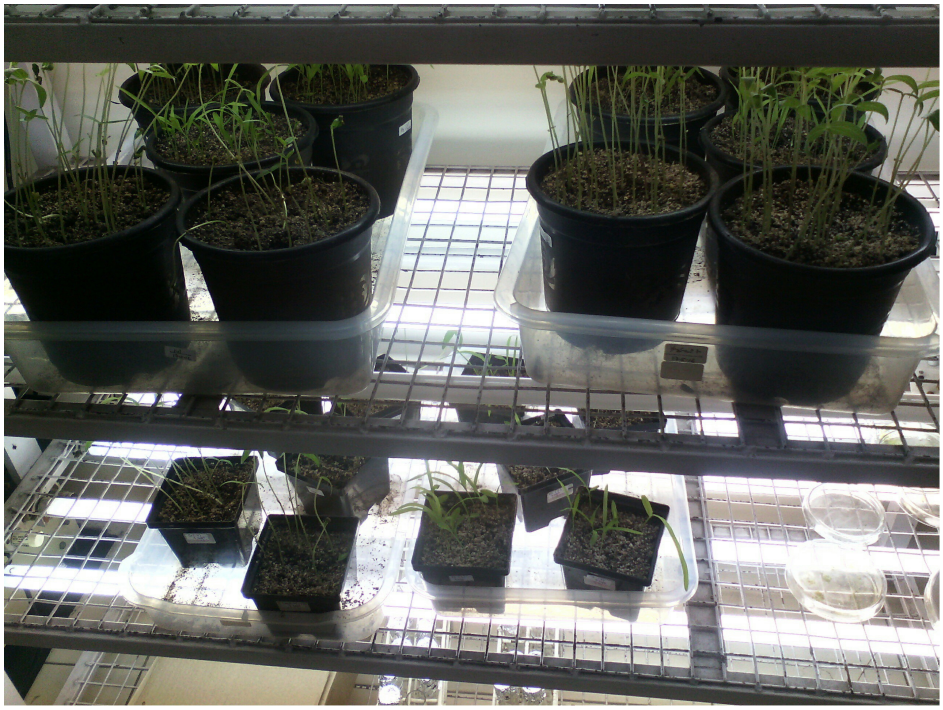
Pots filled with vermiculite and are labelled with different metal ion concentration i.e. 10ppm,50ppm,100ppm.

Seedling of *Sorghum bicolor* after 2 days (seed first grown in agar medium then transferred to the pots) , irrigated with different concentration of Cr i.e. 10ppm,50ppm and 100ppm.



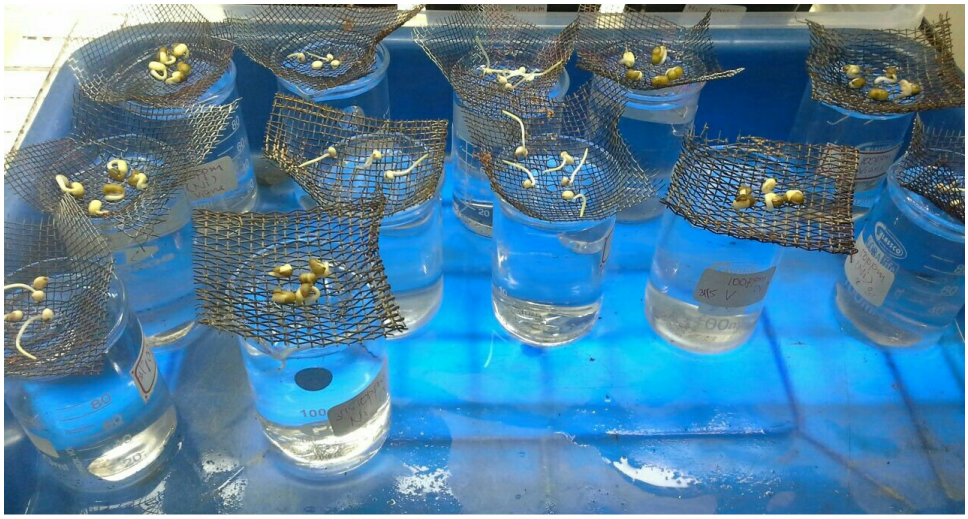
Vigna radiata growth in 100ppm of heavy metal Nickel after 5 days of germination.

Seedling growth of *Sorghum* and *Vigna* after 15 days, irrigated with 100ppm of metal ion concentration.



Larger and smaller pots having seedlings of *Sorghum* and *Vigna* (after 15 days of growth) placed in culture lab under fluorescent light and controlled temperature for proper growth

Dried samples of roots and shoots of *Sorghum* and *Vigna* placed in crucibles for converting them into ash in muffle furnace.



Seeds of *Vigna* placed on the wire gauge with holes in it so that the roots of the germinating seedling could easily reach and absorb the nutrient from the solution beneath it containing the metal ion at different concentration.

Three nucleus (one small and two large) are observed to be present in maximum cell of the meristematic region of roots of *Sorghum* and *Vigna*

16. Annexure/Any other information