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RISK OF HEAVY METAL POISONING VIA *AMARANTHUS VIRIDIS* GROWING IN POTENTIALLY CONTAMINATED SITE

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ABSTRACT

Heavy Metal toxicity is a subject of great concern, it has adverse effects on human health and environment, and leads to possible organ damage and cancer. Since these heavy metals are non-biodegradable, they enter the food chain and through bio-magnification cause severe damage. A possible solution to this problem is to use bio-remediation, here *Amaranthus viridis* was taken as the experimental model, and Dhapa(East Kolkata wetland) soil was used to grow the organism, and the bio-remediation capacity was measured.

KEYWORDS: Potential, Carcinogens, Xenobiotics, Environment, non-biodegradable.

INTRODUCTION

One of the greatest environmental concerns all over the world is heavy metals and its toxicity. They are naturally occurring elements. They have an atomic weight and density about 5 times greater than water. Their multiple industrial, agricultural, medical and technological applications have led to their wide distribution in the environment, raising concerns over their potential effects on human health and our environment.

The contamination poses a great deal of health risk in growing children including cancer, mutation, organ damage, endocrine disruption and also neurological and behavioral changes. Thus remediation of heavy metals has become a recently active field of research. On the lowest exposure these heavy metals are known to act as teratogens and thus have been termed as human carcinogens according to the U.S Environmental Protection Agency, and the International Agency for Research on Cancer.

Since these heavy metals are non-biodegradable, they gain entry into the food chain. Thus a higher concentration of heavy metal in soil reflects on a higher concentration of its accumulation into plants and eventually into human and animal bodies. The ability of these plants to absorb and accumulate these xenobiotics makes them indicators of environmental pollution and is thus also unfit for consumption.

For this paper *Amaranthus viridis* as the experimental model was taken. *Amaranthus* is a commonly available plant in West Bengal and it grows fairly fast in temperate climate (37°C). The plant with its fast growing nature at a maintainable climate and its widespread distribution through West Bengal was suitable for the experimentation. The soil used was collected from Dhapa (East Calcutta Wetland). Dhapa is a landfill site where the solid wastes of the city Kolkata are dumped. Since the wastes are not segregated according to their biodegradation capability thus it is very common to find heavy metal contamination in this area. Thus for Dhapa soil was an ideal choice for our assay.

MATERIALS AND METHODOLOGIES

Experimental Set-up

The following treatments were undertaken in 4 different experimental set-ups:

1. Control- Sandy Loam (Soil: Sand = 2:1)
2. Treatment 1- Sandy loam + PGPR (*Bacillus subtilis*)
3. Treatment 2- Soil obtained from **Dhapa**
4. Treatment 3- Soil obtained from **Dhapa** + PGPR (*Bacillus subtilis*)

These set ups were prepared and maintained. The seeds of *Amaranthus viridis* were sown and each set up was duplicated.

1.1 Physical characteristics of the soil samples

The soil sample was taken and the physical caracteristics was seen on the basis of Electrical conductance (EC) and pH.

1.2 Studying the interactions between the micro-organisms

- PGPR (*Bacillus subtilis*) and microbes in the sandy loam
- PGPR (*Bacillus subtilis*) and microbes in the Dhapa soil

Procedure

1. The interaction between the organisms used in the treatment was observed by conducting cup-plate assay.
2. Two Nutrient agar media plates were prepared.
3. 0.1 gm/10 ml suspension of Sandy loam and Dhapa soil were prepared.
4. For observing interaction between organisms in Sandy loam and Dhapa soil and PGPR (*Bacillus subtilis*), PGPR (*Bacillus subtilis*) was spread plated on the agar while the soil suspension (either Sandy loam or Dhapa soil) was added in the cups made on the agar.
5. These plates were then incubated for 24 hours at 37°C

1.3 Metal concentration in soil sample

1. 1 gm of soil sample was taken and dried in hot air oven for 48 hours.
2. Then the sample was mixed with 5ml of 2% HNO_3
3. The mixture was poured in a crucible, kept on a hot plate and boiled for about 5 minutes.
4. The remaining sample was the filtered using a filter paper and the sample solution volume was made 10 ml using Milipore water.
5. Now atomic spectroscopy was performed with both of these sample solutions i.e Sandy Loam and Dhapa soil
6. For each of these samples there was a duplicate prepared.
7. The samples were prepared to measure 3 heavy metals.

1. **Cadmium**
2. **Nickel**
3. **Lead**

1.4 Metal tolerance of *Bacillus subtilis*

- **Nickel-** For the formulations of this solution refer to Fig (i)
- **Lead-** For the formulations of this solution refer to Fig (ii)

Procedure

1. Metal solution were prepared in accordance to the above tables
2. 10ml of Nutrient Broth was prepared and 1ml of the metal solutions were added in different test tubes containing the media.
3. The media was autoclaved.
4. 1ml of *Bacillus subtilis*was inoculated in the media containing different metal concentrations.
5. These were incubated at 37°C for 24 hours and later again for 48hours.
6. The O.D. value was measured after incubation of 24hours and 48hours using colorimeter.

1.5 Metal uptake capacity of *Bacillus subtilis*

1. Stock solutions of heavy metals were prepared by using Lead acetate and Nickel sulphate, to attain maximum solubility.
2. The stock solutions were prepared with 1000ppm concentration of respective metals in deionised water.
3. The biosorption of metals by isolates were assayed in Erlenmeyer flask containing 90ml of **Metal Biosorption Media**[4] (NaCl- 81.0, MgCl2-7.0, MgSO4·7H2O- 9.6, CaCl2-0.36, KCl- 2.0, NaHCO3-0.06, NaBr- 0.026, yeast extract- 5.0, and glucose- 3.0 g/L), added with 1ml of metal solutions having 1000ppm concentration.
4. To this 10ml of overnight culture of isolates (*Bacillus subtilis*) was added.
5. pH of metal microbe suspension was adjusted to 6.5 ±0.02, to facilitate the maximum solubility of metal irrespective of the optimal pH for the growth of the isolate.
6. The metal microbes suspension was incubated at 40°C under constant stirring at 150 rpm, for 24 hrs.
7. A control without bacterial culture was also maintained.
8. The biosorption potential was measured as amount of metal removed from the medium by estimating the residual metal concentration using Atomic Absorption Spectroscopy (AAS).
9. All the biosorption experiments were carried out in duplicate and average value was taken from them.
10. After incubation, the biosorption of respective metal biosorption by the isolates was measured by removing the cells from the medium by centrifuging at 8000 rpm for 20min.
11. Standard solutions of individual metals were prepared with varying concentrations in milli-pore water.
12. The standard's absorption ofmetal solutions along with experimental set-up was measured by Atomic Absorption Spectroscopy.
13. The supernatant was analysed for residual metal concentration in the bacterial treated and culture free control media.

1.6 Metalconcentration in *Amaranthus viridis* leaves

1. 1gm of dry leaf was taken after drying it in the hot air oven for 24hours.
2. Then the sample was mixed with 5ml of 2% HNO3.
3. The mixture was poured in a crucible and kept on hot plate and boiled for 5minutes.
4. The remaining sample solution was filtered using filter paper and the sample solution volume was made 10ml using Millipore water.

5. Now atomic spectrometry was performed with three sample solutions i.e. sandy loam control plant, dhapa soil control plant and dhapa soil PGPR treated plant.
6. This was performed at 6th week and also 8th week.
7. The samples were prepared to measure 2 heavy metals- **Nickel and Lead**

RESULTS

2.2 Physical characterization of the soil sample

Observation

The pH of the Sandy loam was 6.9 and the Dhapa soil is 6.7 respectively. The electrical conductance or the EC being 782µs and 952µs for Sandy loam and Dhapa soil respectively.

2.3 Interaction among the microbes in the soil samples and the PGPR

Observation

No antagonistic interaction was seen in between

a) PGPR (*Bacillus subtilis*) and the micro-organisms in the sandy loam
b) PGPR (*Bacillus subtilis*) and the micro-organisms in the Dhapa soil

2.4 Metal concentration in soil samples

Before sowing the seeds

a) Nickel Concentration: The mean concentration was obtained as 0.288mg/ml in Sandy loam and 0.65mg/ml in Dhapa soil. Refer to fig (iii).

b) Lead Concentration: The mean concentration was obtained as 0.215mg/ml in Sandy loam and 6.44 mg/ml in Dhapa soil. Refer to fig (iii)

6th week results of metal concentration

a) Nickel Concentration: Sandy loam- 0.107mg/ml
Sandy loam + PGPR- 0.094mg/ml
Dhapa soil- 0.567mg/ml
Dhapa soil + PGPR- 0.347mg/ml (Refer fig (v)

b) Lead concentration: Sandy loam- 0.056mg/ml
Sandy loam + PGPR- 0.046mg/ml
Dhapa soil - 3.468mg/ml
Dhapa soil + PGPR- 1.734mg/ml (Refer fig vi))

8th week results of metal concentration

a) Nickel Concentration: Sandy loam- 0.092mg/ml
Sandy loam + PGPR- 0.056mg/ml
Dhapa soil- 0.178mg/ml
Dhapa soil + PGPR- 0.123mg/ml (Refer fig (vii)

b) Lead Concentration: Sandy loam- 0.041mg/ml
Sandy loam + PGPR- 0.023mg/ml
Dhapa soil- 3.178mg/ml
Dhapa soil + PGPR- 1.237mg/ml (Refer fig (viii))

Nickel: The mean concentration of metal uptake capacity is 2.4mg/lt. Refer to fig (xiii)
Lead: The mean concentration of metal uptake capacity is 2.4mg/lt. Refer to fig (xiv)

2.6 Metal concentration in *Amaranthus viridis* leaves

6th week

a) Nickel Concentration: Sandy loam- 0.121mg/ml
Dhapa soil- 0.187mg/ml
Dhapa soil + PGPR- 0.227mg/ml (Refer fig (xv))

b) Lead Concentration: Sandy loam- 0.343mg/ml
Dhapa soil- 0.367mg/ml
Dhapa soil + PGPR- 0.313mg/ml (Refer fig (xv))

8th week

a) Nickel Concentration: Dhapa soil- 0.529mg/ml
Dhapa soil + PGPR- 0.721mg/ml (Refer fig ())

a) Lead Concentration: Dhapa soil- 0.678mg/ml
Dhapa soil + PGPR- 0.895mg/ml (Refer fig ())

Fig (i) Formulation of Nickel Solution.

Nickel concentration in ppm	Volume of Ni in mg added to 100 ml of distilled water
Control	0.00
25ppm	0.66
50ppm	1.32
100ppm	2.64
300ppm	7.91
500ppm	13.18
600ppm	15.84
800ppm	21.12
1000ppm	26.40
1500ppm	39.60
2000ppm	52.80
2500ppm	66.00
Lead concentration in ppm	**Volume of Pb in mg added to 100ml of distilled water**
Control	0.00
25ppm	0.34
50ppm	0.67
100ppm	1.34
200ppm	2.64
300ppm	4.03
500ppm	6.71
600ppm	8.05
800ppm	10.72
1000ppm	13.40

Fig (ii) Formulation of lead solution.

Fig (iii) Nickel (Ni) concentration in (mg/lt).

Set	Sandy Loam Ni (mg/lt)	Dhapa soil Ni (mg/lt)
I	0.37+/-0.0022	0.629+/-0.0037
II	0.206+/-0.0012	0.671+/-0.0040
mean	0.288+/-0.0014	0.65+/-0.0041

Fig (iv) Lead (Pb) concentration in (mg/lt).

Set	Sandy loam Pb (mg/lt)	Dhapa soil Pb (mg/lt)
I	0.156+/-0.0003	5.516+/-0.56
II	0.274+/-0.0011	7.37+/-0.79
mean	0.215+/-0.0023	6.44+/-0.63

Fig (v) 6th week metal concentration in soil.

Sample	Nickel conc (mg/lt)	Lead conc (mg/lt)
Sandy loam (control)	0.107+/-0.001	0.056+/-0.00031
Sandy loam (PGPR)	0.094+/-0.0011	0.046+/-0.00027
Dhapa soil (control)	0.567+/-0.0032	3.468+/-0.23000
Dhapa soil (PGPR)	0.347+/-0.0015	1.734+/-0.04500

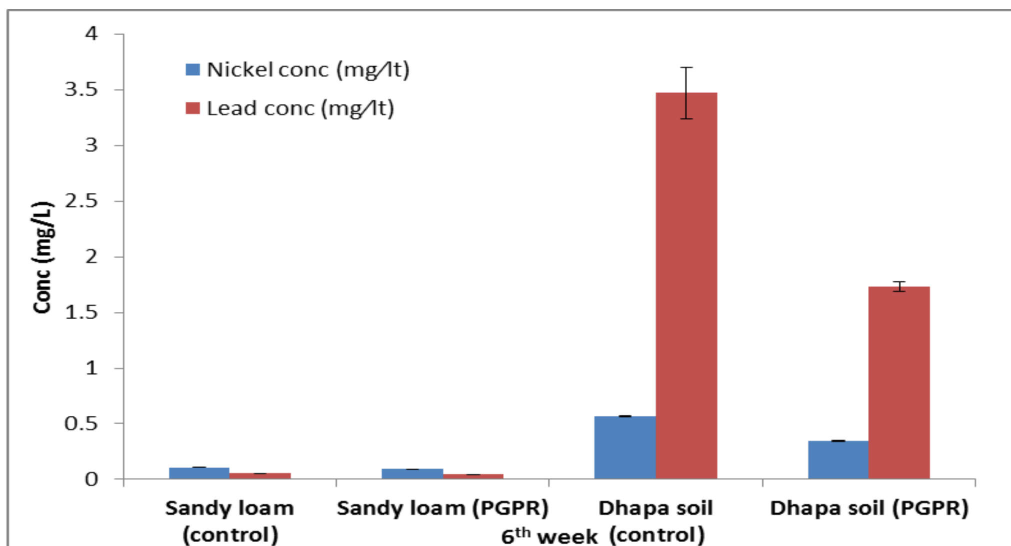


Fig (vi) 6th week metal concentration in soil.

Fig(vii): 8th week metal concentration in soil.

Sample	Nickel conc (mg/lt)	Lead conc (mg/lt)
Sandy loam (control)	0.092+/-0.0023	0.041+/-0.0005
Sandy loam (PGPR)	0.056+/-0.0055	0.023+/-0.0012
Dhapa soil (control)	0.178+/-0.0580	3.178+/-0.22
Dhapa soil (PGPR)	0.123+/-0.0001	1.237+/-0.0440

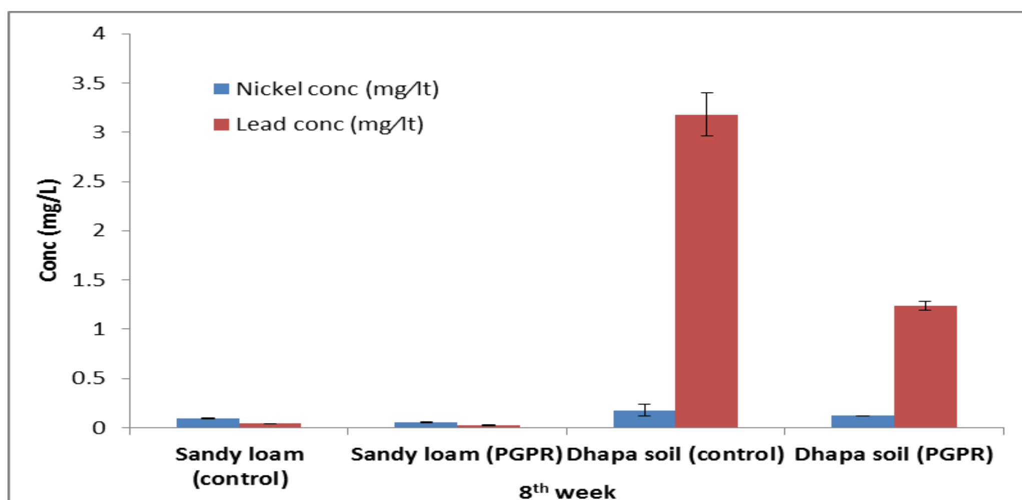


Fig (viii) 8th week metal concentration in soil.

Fig (ix): Metal tolerance of *B.subtilis* for Nickel.

Ni conc (ppm)	24hr O.D (600nm)	48hr O.D (600nm)
Control	0.38	0.85
25	0.41	0.545
50	0.44	0.575
100	0.46	0.56
300	0.49	0.58
500	0.525	0.56
600	0.51	0.59
800	0.53	0.593
1000	0.532	0.597
1500	0.54	0.589
2000	0.48	0.55
2500	0.44	0.51

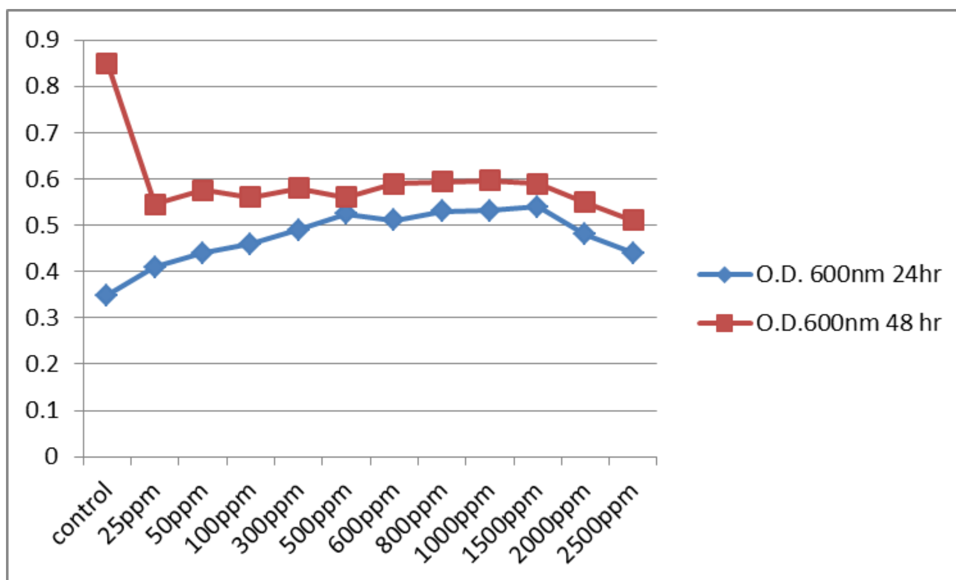
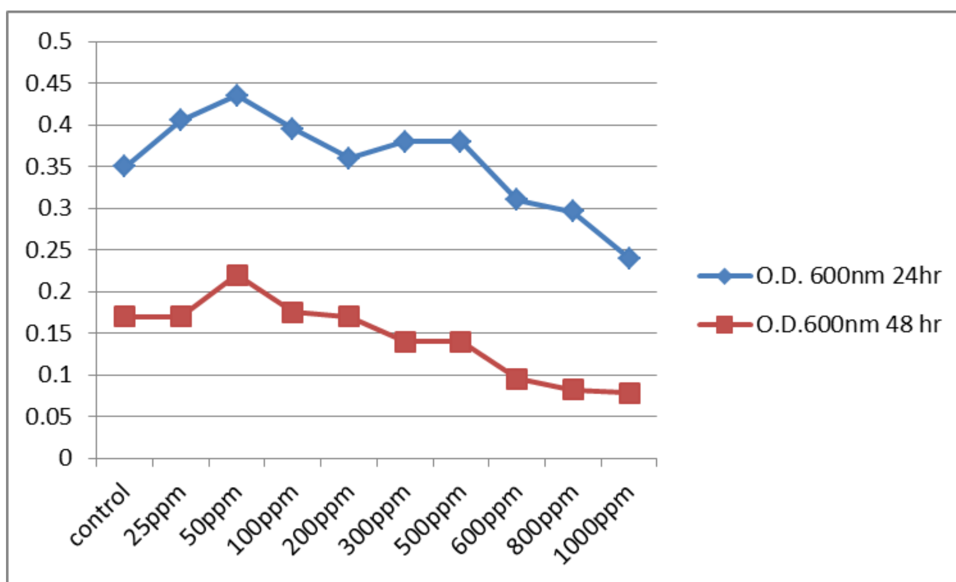


Fig (x) Metal tolerance of *B.subtilis* for Nickel.

Fig (xi) Metal tolerance of *B.subtilis* for Lead.

Pb conc (ppm)	24hr O.D (600nm)	48hr O.D (600nm)
Control	0.35	0.17
25	0.405	0.17
50	0.435	0.22
100	0.395	0.175
200	0.36	0.17
300	0.38	0.14
500	0.38	0.14
600	0.31	0.095
800	0.296	0.082
1000	0.24	0.078



Fig(xii): Metal tolerance of *B.subtilis* for Lead.

Fig(xiii): Metal uptake of *B.subtilis* for Nickel.

Set	Nickel concentration (mg/lt)
I	2.5+/-0.1101
II	2.3+/-0.12
mean	2.4+/-0.11

Fig(xiv): Metal uptake of B.subtilis for Lead.

Set	Lead concentration (mg/lt)
I	1.2+/-0.04331
II	0.8+/-0.03580
mean	1.0+/-0.4103

Fig(xv): Metal concentration in 6th week plants.

Sample	Nickel conc (mg/lt)	Lead conc (mg/lt)
Sandy Loam (control)	0.121+/-0.0048	0.343+/-0.0042
Dhapa soil (control)	0.187+/-0.0043	0.367+/-0.0042
Dhapa soil (PGPR)	0.227+/-0.0044	0.313+/-0.0047

Fig(xvi): Metal concentration in 8th week plants.

Sample	Nickel conc (mg/lt)	Lead conc (mg/lt)
Non-PGPR treated	0.529+/-0.0053	0.678+/-0.0061
PGPR treated	0.721+/-0.0071	0.895+/-0.0079

1ST Week

Sandy Loam (Control)

Dhapa (Control)

3rd Week

Sandy Loam (Control)

Sandy Loam (PGPR Treated)

Dhapa (Control)

Dhapa (PGPR treated)

6TH Week

Sandy Loam (Control)

Sandy loam (PGPR treated)

Dhapa (Control)

Dhapa (PGPR treated)

DISCUSSION

From the above experiments it was possible to conclude that Amaranthus viridis being a leafy vegetable could accumate these heavy metals in their plant parts On the other hand the plant growth promoting rhizobacteria *Bacillus subtils*which could effectively remediate the heavy metal toxicity from soil. Inspite of giving the proper PGPR, Amaranthus viridis accumulated as high as 0.721mg/ml of Nickel and 0.895mg/ml of lead. *B.subtilis* have Nickel tolerance till 1500ppm and Lead tolerance till 50 ppm. But on increase in concentration to these heavy metals they show intolerance and thus they get non-viable. Thus with application of PGPR, does not show the plants being free of toxicity and thus can be said that leafy vegetables grown in Dhapa soil are not suitable for consumption. Our result is according to the findings by[1] where there was higher concentration of Cadmium and lead. Melon vegetables demonstrated a relatively low capacity for accumulating the heavy metal studied. According to Smirjakova et al, 2005,[2] heavy metal such as cadmium is taken up by the roots which then passes to the edible parts of the plant. In the fauna they could be accumulated in the milk of lactating mammals and also in their fatty tissues. So mankind is at a high chance of being exposed to cadmium toxicity through their above mentioned foodchain. A study conducted by Saper et al,[3] on Ayurvedic herbal medicinal products manufactured in South Asia and sold in Boston areas revealed that out of 70 consumable drugs, 14 contained harmful levels of metals such as Lead, Mercury and Arsenic. So an awareness was created that the users of Ayurvedic medicinal products are at a high risk of heavy metal contamination and thus strict testing of heavy metal concentrations for these products was mandatory. Thus from the assay it could be concluded that consumption of leafy vegetable from such polluted land is not considered safe and should be discarded from the diet completely for a healthy lifestyle.

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