

Influence of Industrial effluent and Agnihotra ash on *Azadirachta indica* growth: A case study

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ABSTRACT

In this current study, *Azadirachta indica* native plant to Indian sub-continent was selected as the subject to screen the effect of industrial effluents as well as Agnihotra ash on its growth. Effluents from various industries were used. Control plant was grown on tap water. Rest of the saplings were grown on effluents procured from other sources. All these were compared to the plant grown on Agnihotra ash. Based on the observations, it was concluded that the Agnihotra ash based plant growth was superior of all.

1. Introduction

Potable source of water is crucial for the sustenance of life. Water is naturally obtained from the environment and used for agricultural, industrial and various domestic purposes. The consumed water is often discharged to nearby water bodies either with minimal or no treatment. Owing to the surge in population growth as well as the urbanization, there has been a concomitant increase in the volume of water used and consequently the quantity and quality of wastewater thrown into the water bodies in the region. These wastewaters contain toxic contaminants, which if continuously discharged to water streams might seriously affect the marine ecosystem, fresh water reservoirs and eventually pose threat to human existence [1-2].

In context of industrialization, water resources come in contact with processing materials/equipment and the eventually gets contaminated with traces of toxic metals. According to the World Health Organization recommendations, the metals of most immediate concern are cadmium, chromium, cobalt, mercury and zinc. Such heavy metals bio-accumulate readily in marine organisms and subsequently consumed by humans. Since these are carcinogenic, these interfere with growth or metabolism of cells in the body. Current techniques for the treatment of these heavy metals are based on precipitating the metals out of the wastewater as their hydroxides, by the addition of lime or caustic. Precipitates are either concentrated or just land-filled [3]. Certainly, the problem does not get solved but gets transferred from one medium to the other. This highlights to have a sustainable solution to the wastewater problem [4].

The Neem tree (*Azadirachta indica*) of the family *meliaceae* is native to the Indian sub-continent. Since ancient times, all the tree components have been in use to treat a number of afflictions as well as the household pesticide. A plethora of medicinal and germicidal properties has been attributed to it. The Neem tree and Agnihotra ash serve as a natural air purifier has been recommended as an effective way to combat traffic-related pollution [5-6]. The tree not only helps in the growth of other trees in barren land by improving soil fertility but also exhibits scavenging properties.

In the current study, the tree saplings of *Azadirachta indica* were employed as phytoremediators. Further, the biochemical changes occurred after 2 months of exposure of plants to the wastewater were compared with plants grown on Agnihotra ash [7-9].

1. Methodology

Several industries were contacted and visited on a number of occasions to collect water samples.

Wastewater samples used in the investigations were collected from three different locations pertaining to pharmaceutical company, AC manufacturing firm and textile industry. In all cases, samples were collected and stored in pre cleaned plastic containers that had been rinsed thoroughly with dilute nitric acid in order to remove all heavy metals and organic chemical residues. 5 identical saplings of Neem plant were procured along with the soil from Nursery, Chandigarh. Each plant sapling was 4 months old at the time of analysis. Each plant sample was named as NS1, NS2, NS3, NS4 and NS5. The pots were periodically watered by avoiding running down of water from the pots. The plants were allowed to grow in outdoor conditions under identical conditions i.e. amount of water, soil type, sunlight, temperature for 60 days. All saplings were given different treatments as described in Table 1. Soil samples were analyzed after 50 days. Subsequent shoot samples were analyzed after 60 days. Shoot, root length, leaf size and area were also measured.

The samples were grown in soil comprised of red soil (60%), manure (20%), sand (10%) and diammonium phosphate in trace amounts. A number of different analyses were carried out on the wastewater, soil samples and on the plant samples. Heavy metal concentrations in wastewater and in soil were determined for all samples by Wavelength Dispersed X-Ray fluorescence (WD-XRF). For plant sample analysis, atomic absorption spectroscopy (AAS) was used. Following sample preparation were used as required for different instruments:

Table 1 Various treatments on the saplings

Sample Name	Irrigated with	Quantity (per day)
NS1	Tap water	300ml
NS2	Treated wastewater of Pharmaceutical company	300ml
NS3	Treated waste water of AC firm	300ml
NS4	Treated wastewater of Textile industry	300ml
NS5	Tap water and Agnihotra ash	20 grams of agnihotra ash, 300ml tap water

Sample preparation for WD-XRF:

10 ml sample was taken in plastic cup holders with Mylar film of thickness 0.6 microns as base. The total run time was for 2-3 minutes. Helium gas was used to cool the sample.

Soil sample was mixed binder tablets (1:1). Binder tablets were made of Cellulose and Poly Vinyl Alcohol (PVA). The total weight of the sample became 11.27 grams. The soil and binder were homogenized to a fine powder form in a Milling machine. 10 gram of the powder was taken, and then using Hydraulic press, under pressure of 15 tonnes, pellets were formed. Diameter and thickness of pellet were 34mm and 2mm respectively. Run time was 37 minutes.

Ash was mixed with Binder (4:1; Cellulose powder) and pellets were formed.

Sample preparation for AAS:

Sample was digested by dissolving 0.10 g in a mixture of 25 mL of concentrated nitric acid and 10 mL of concentrated sulfuric acid in a beaker under sterile conditions. The sample was then heated in order to get all material to dissolve. Once

dissolved, the mixture was added slowly to 50 mL of distilled water in a 100 mL volumetric flask. The beaker was rinsed several times to ensure that all material has been transferred.

10 gram soil was diluted with 50 mL double distilled water and was kept for 2 hours. Then using pH meter, the pH was determined.

2. Results and Discussions

The Agnihotra ash contains trace elements which improves the nutrient content of the soil. Due to the presence of oxides, it increases the pH of the soil sample but still successfully improves the plant growth. The soil contains a high amount of lead (21ppm), which is quite high than the safe limit (8ppm). As these are also affecting the plants in some form or the other form. Cr content is also very high (88ppm) in the soil, which is then accumulated in to the shoots of the plants. The soil also contains Co (88ppm) which is also found to deteriorate the plant's growth.

Table 2 Concentration of heavy metals in the water samples

Heavy metal Conc (ppm)	NS2	NS3	NS4	NS5
Chromium	4	10	1	4
Cadmium	0	0	0	0
Lead	0	0	0	0

It was speculated that lead and cadmium may be present in very small concentrations i.e. in ppb, as these could not be estimated through the XRF instrument.

Table 3 The physiological properties of the samples

Parameters	NS1	NS2	NS3	NS4	NS5
pH	7	7.95	6.05	7.53	9.45
Leaf length	4.12	3.91	3.34	4.25	4.42
Odour of water	-	Extremely Foul	Foul	-	-
Color of leaves	green	Yellow	Brownish green	Light green	green
Increment in Shoot length(cm)	22.6	20.6	18.34	22.21	29.56
Root length(cm)	37.49	37.58	36.98	37.32	38.14
No. of leaves	29	17	32	28	30
Chromium uptake in shoots (ppm)	-	1.8	1.4	-	-

The average shoot length at the start of irrigation was approximately 67 cm. Agnihotra ash increases the pH of the soil. Even though low pH favors the growth of the plant. Despite this fact, N3 (pH: 6.05 is the worst effected) whereas

N5 (pH: 9.45) has shown the best growth. There was increase in leaf size, shoot length, and the color and texture of the leaves was also significantly improved than the control plant. Leaf length of N5 was also found to be the highest (4.42 cm) as

compared to N1 (4.12). Hence, Agnihotra ash significantly increased the leaf size [8-9]. Although the height of shoots and roots of all the plant samples were not precisely similar, yet there was a noticeable difference in the shoot length in all the samples. The leaves of N2 were falling off at a high rate. There was a significant amount of graining and the color also started to fade after 40 days. The water was little green in colour and with high amount of bad odour. In N3, there was almost a standstill growth for the first 3 weeks. After that it slowly started to grow, with severe graining on its leaves. The water was also very pungent smelling. N4 showed very little change from the control plant. N4 was initially taller than the rest of the samples. The pH was on the higher side as well though the leaf and shoot length was lower as compared to its root length.

Further, the uptake of Cr by plants established that the same could be used as an alternative strategy to combat heavy metal contamination at the source point [7].

3. Conclusion

Even as the amount of chromium, according to the standards was within safe limits, its effect was still detrimental to the physiological parameters of the plant. Hence, the industrial effluent needs to be treated. Moreover, Agnihotra ash improved the physical parameters of the plant. The genetic studies of plant such as its RNA activity can be carried out to further analyse the effect of Agnihotra ash and chromium on the neem plant.

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