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HEAVY METAL (AL_2O_3) TOLERANCE OF SOIL BACTERIA ISOLATED FROM PLANT RHIZOSPHERE REGION OF *VIGNA MUNGO* L.

Dikshyashree Mohapatra, M. Mohapatro, G. Sabat, R. Padhy, Sarita P. Nayak, B. K. Mohanty

PG Department of Botany and Biotechnology, Khallikote Autonomous College, Berhampur, Odisha, India.

ABSTRACT: A total of four bacterial strains (namely Isolate 1 to Isolate-4) were isolated from sample collected at the dumping site nearer to a garage, Berhampur. The bacterial resistance was studied with the treatment of varied concentrations of Aluminum. It was observed that, Isolate -3 showed highest tolerance in all Al_2O_3 concentrations (25 ppm, 50ppm, 100ppm, 150ppm) but showed highest tolerance in 100ppm concentration of Al_2O_3. Isolate 1 showed highest tolerance in 50ppm concentration of Al_2O_3. Isolate 4 showed highest tolerance in 25ppm concentration of Al_2O_3 . Isolate 2 showed highest concentration in 50ppm concentration of Al_2O_3 but showed weak growth with increased concentration. Antibiotic sensitivity test was conducted to check against four antibiotics- Streptomycin, Gentamicin, Cloxacilin, Rifampicin – using discs on the nutrient agar plates and it was found that, the highly aluminum resistant bacteria (Isolate-3) showed high resistance to antibiotic Cloxacilin and sensitive to Gentamicin, Streptomycin and Rifampicin but more sensitive to Gentamicin.

KEYWORDS: Aluminum, *Vigna mungo* L., Bacteria, antibiotic

Corresponding Author: Dr. B. K. Mohanty* Ph.D.

PG Department Of Botany &Biotechnology, Khallikote Autonomous College, Berhampur, Odisha, India. Email Address:mohantysir57@yahoo.com

1. INTRODUCTION

Heavy metals have an adverse effect on human physiology and other biological systems [1, 2]. They show a great affinity for other elements such as sulphur disrupting enzyme functions in living cells by forming bonds with this group. Cd has no essential biological function and is thus highly toxic to living organisms. Chronic exposure to cadmium in humans has several toxic effects, such as high

blood pressure, kidney, lung, liver and testes damage [3,4, 5]. Aluminum (Al) toxicity is one of the major constraints on crop productivity on acid soils, which occur on up to 40% of the arable lands of the world. Al is the third most abundant element in the earth's crust and is toxic to plants when solubilised into soil solution at acidic pH values [6]. Soils are becoming more acidic by certain farming practices, for example the application of ammonium-based fertilizers, and accumulation of organic matter [7]. Al toxicity is considered to be a complex of nutritional disorders of growth and development of plants, which may be manifested as a deficiency of essential nutrients like calcium, magnesium, iron or molybdenum; decreased availability of phosphorus or as toxicity of Mn and H^+ .The primary response to Al stress in plants occurs in roots, as reduced elongation at the tip, followed by swelling and distortion of differentiated cells, as well as root discoloration [8] Within meristematic and root cap cells, Al toxicity is associated with an increased vacuolation and turnover of starch grains, as well as disruption of dictyosomes and their secretory function. Al toxicity inhibits root cell division and elongation, thus reducing water and nutrient uptake, consequently resulting in poorer plant growth and yield [9,10,11]. Al toxicity also limits both rooting depth and degree of root branching demonstrated that there are two responses to Al: an initial acute inhibition of growth that is followed by a later chronic Al effect on root growth. Al toxicity decreases drought tolerance and the use of subsoil nutrients [12]. From phylogenetic diversity in soil it is estimated that a gram of soil contains approximately 6000 species [13, 14]. Microbial communities are constituted by structural clusters of microbial species, each playing different and complementary roles. The environmental stress caused by heavy metals, generally decreases the diversity and activity of soil bacterial populations leading to a reduction of the total microbial biomass, decrease in numbers of specific populations such as rhizobia and a shift in microbial community structure [15, 16]. The response of the bacterial populations to heavy metal contamination depends on the concentration and bioavailability of metals itself and is dependent by multiple factors such as the type of metal and microbial species [17]. The presence of different metals together may also have greater adverse effects on the soil microbial biomass/activity and diversity than those caused by a single metal at high concentrations [18] Low concentrations of certain metals such as zinc, copper, cobalt and nickel are essential for the metabolic activity of bacterial cells. Other metals like Pb, Cd, Hg and Cr have no known effects on cellular activity and are cytotoxic [19]. Arsenic resistant organisms were isolated as expected from arsenic contaminated environments, but laboratory strains of bacteria such as *Escherichia coli, Pseudomonas aeruginosa* show resistance to high levels of As [20]. Aluminium (Al), the most abundant metal and the third most common element in the earth's crust [6, 21]is present in all soils [22]. It makes up approximately 7.1% of the solid matter in an average soil. Aluminum becomes more toxic to many plants at concentrations greater than 2-3 ppm in acidic soils therefore the potential for soils to be Al-toxic is considerable [23]. Al toxicity is a recognised widespread problem in biology. Even an Al^{3+} concentration of about 1ppm in solution can inhibit

the growth of plant roots. Therefore, ionic Al is an important limiting factor in the growth of many plants in various acid soils. Aluminium is harmful to the activities of many soil microorganisms. The only known bacteria that can tolerate or perhaps prefer Al, are the Al-corrosive bacteria. Al Toxicity is a recognized widespread problem in biology. Even an Al^{3+} concentration of about 1ppm in solution can inhibit the growth of plant roots. Therefore, Ionic Al is an important limiting factor in the growth of many plants in various acid soils [24]. Aluminium is harmful to the activities of many soil microorganisms [24, 25]. Arsenic resistant organisms were isolated as expected from arsenic contaminated environments, but laboratory strains of bacteria such as *Escherichia coli, Pseudomonas aeroginosa* show resistance to high levels of As [26].

2. MATERIALS AND METHODS

Collection of Soil sample:

Soil samples were collected from metal contaminated area located near a dumping site of Berhampur, Ganjam, Odisha. Sterile digging tools were used to collect soil samples in range of 10cm to 20cm below the soil surface.

Selection of seeds:

The seeds of *Vigna mungo* L. (variety- B_3-8-8-Prasad) were collected from Centre for Pulse Research (CPR) Ratanpur, Berhampur, Ganjam, and Odisha. Healthy, disease free and uniform size seeds were selected. Nutrient agar media and Nutrient Broth Media were used throughout the experiment both for culturing and maintaining the test bacteria for bioassay study.

Soil Analysis:

The collected soil sample was analyzed and composition of soil was tested to find out the amount of Organic carbon, p^H, available P_2O_5, Available potash in the soil. 500gm of contaminated soil samples were taken in a pot. 10-15 seeds of *Vigna mungo* L. were sowed in the pot. In a particular interval of time pot was properly watered and placed in the garden of botany department, Khallikote Autonomous College, Berhampur, Ganjam, (Odisha).

Germination of Seeds:

After 10 days seeds were germinated and the roots developed. Plants were rooted out from the pot and 1gm of soil sample was collected from the rhizosphere region. To isolate the bacteria serial dilution technique was done.

Preparation of soil sample:

Serial dilution:

For serial dilution technique, along with one test tube of 10ml, 10 test tubes of 9ml were taken. 1gm of fresh soil sample was added in 10ml of distilled water and also marked. 1ml of the suspended soil samples was added tube 9ml of distilled water to make a one in 10 dilution (10^{-1}), and then 1ml of this dilution was added to 9ml of distilled water to make a one in 100 dilution (10^{-2}). This procedure was repeated until 10^{-10} dilution was reached.

Isolation of Bacterial Strains:

A total of four bacterial strains were isolated from sample collected at the dumping site nearer to a garage, Berhampur. Based on the preliminary morphological examination of bacterial strains on nutrient agar, most of bacterial isolates revealed formed whitish, entire and irregular colonies. Some isolates form yellow and cream colored colonies. Besides, microscopic analysis showed that most isolates were rod shaped Gram positive bacteria.

Heavy metal Stock Solution:

Heavy metal stock solution was prepared by diluting the appropriate weight of metal (Al_2O_3) in broth solutions of varying concentrations (25ppm, 50ppm, 100ppm, 150ppm) of Al_2O_3 and sterilized by autoclaving at 121^0C for 15 minutes.

Measurement Level of Bacterial Resistance:

Heavy Metal (Al_2O_3) Resistant:

For each isolate five test tubes were taken along with 10ml of nutrient broth. To each test tube 200µl of specific bacterial stock solution and 200µl of heavy metal stock solution of specific concentrations were added. Then they were kept in the shaker at 37^0c for 24hours. O.D values were taken at 600nm by the help of a spectrophotometer. The bacterial colonies that grew on the highest concentration of Al_2O_3 metal supplements or high level with sensitivity of bacteria control were observed.

Antibiotic Sensitivity Test:

Antibiotic Sensitivity was checked against four antibiotics- Streptomycin, Gentamicin, Cloxacilin, Rifampicin – using discs on the nutrient agar plates after spread plating of each culture. Plates were incubated for 24 hours at 37^0c. The amount of space around every disc indicates the zone of inhibition. In general, larger zones correlate with smaller Minimum Inhibitory Concentration (MIC).

Identification of Bacterial Strains:

Gram Staining:

Staining was carried out by standard procedure of gram staining. The slides were observed under compound microscope (40x magnifications) by examining Gram reaction test and it's morphological appearances such as color and the shape of bacterial colony.

3. RESULTS AND DISCUSSION

Physicochemical properties of soil sample:

Some of the physicochemical properties of the soil sample were determined and observed that it was of neutral soil (pH 6.8) with a relatively high content of potash, organic carbon and organic phosphorus as shown in the table below:

Soil properties	Values	Nature
pH	6.8	Neutral
Electrical conductivity	0.60	Normal
Available phosphorus	72.33	High
Organic carbon	1.3	High
Available potash	375	high

The observed morphological characteristics pertaining to color, shape & elevation were shown in the table below.

Bacterial Isolates	Colour	Shape	Elevation
Isolate-1	Yellow	Circular shiny	Convex
Isolate-2	White	Irregular shiny	Flat
Isolate-3	White	Irregular	Flat
Isolate-4	Cream	Irregular	Flat

Cell Morphology:

Cell morphologies of strains were studied and the observations were described as in the table below:

Cell morphology of isolated strains:

Isolates	Colour	Gram staining	Shape
1	Pink	-ve	Cocci
2	Purple	+ve	Rod
3	Purple	+ve	Rod
4	purple	+ve	Rod

Resistance of bacterial Isolate to Al_2O_3

Isolate 1 showed highest tolerance in 50ppm concentration of Al_2O_3. Isolate 2 showed highest tolerance in 50ppm concentration of Al_2O_3 but showed weak growth with increased concentration. Isolate -3 showed tolerance in all concentrations (25 ppm, 50ppm, 100ppm, 150ppm) but showed highest tolerance in 100ppm concentration of Al_2O_3. Isolate 4 showed highest tolerance in 25ppm concentration of Al_2O_3.

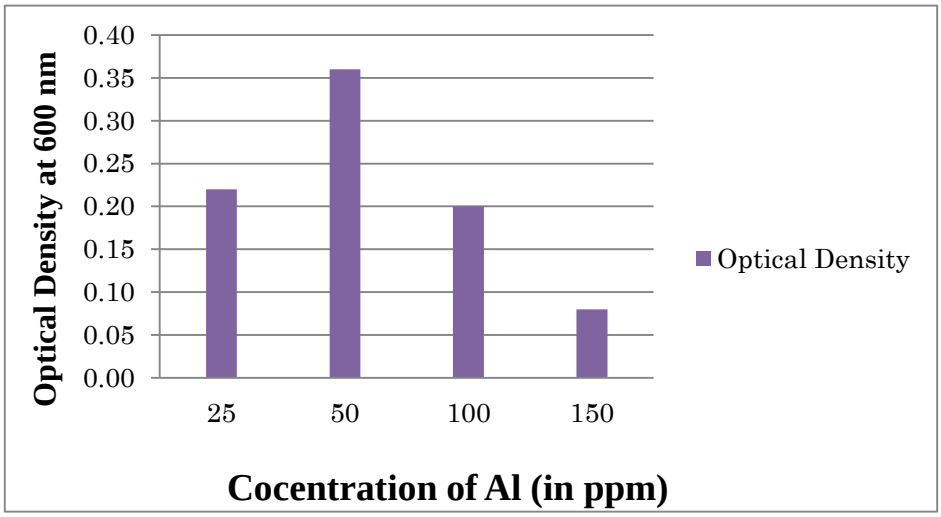

Fig No.1: Resistance of bacterial isolate -1 to Al_2O_3 measured in term of Absorbance at 600 nm against Al Concentration (ppm).

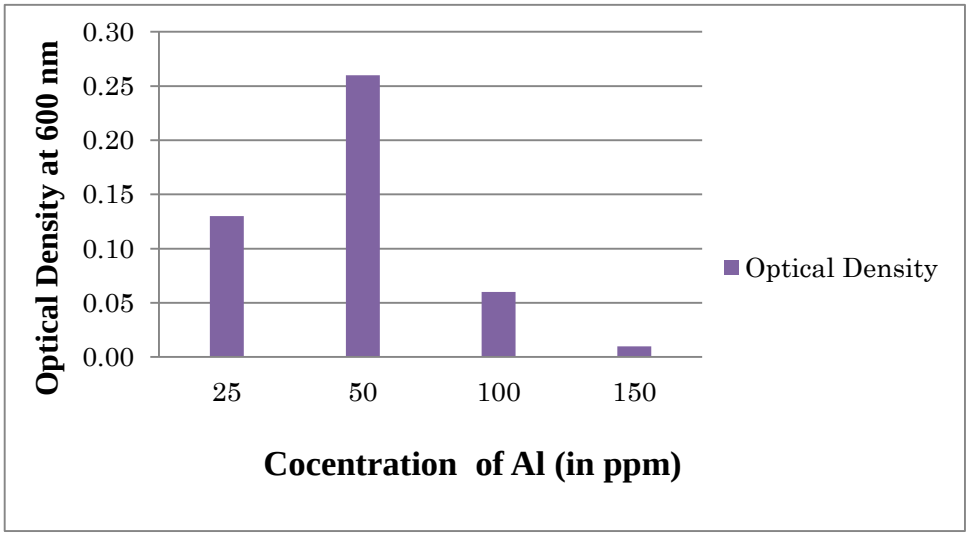

Fig No.2: Resistance of bacterial isolate -2 to Al_2O_3 measured in term of Absorbance at 600 nm against Al Concentration (ppm).

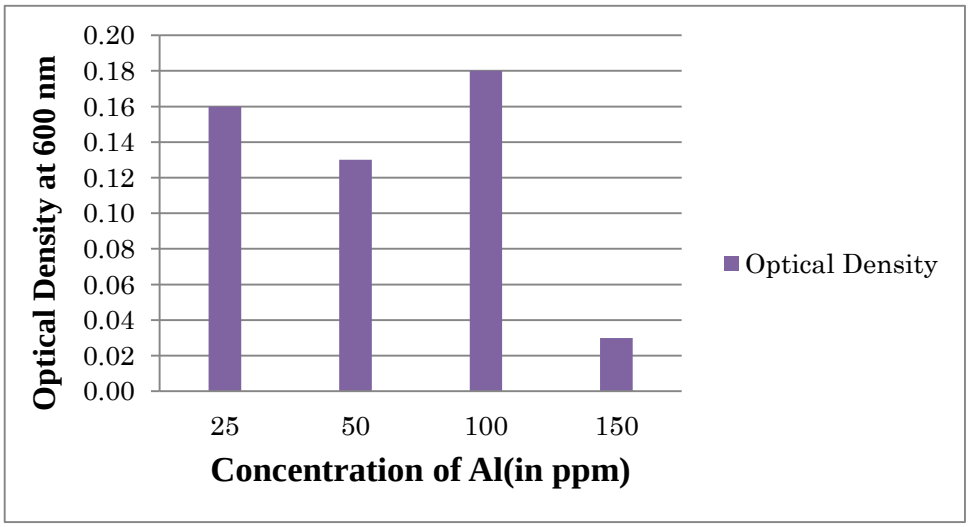

Fig No.3: Resistance of bacterial isolate -3 to Al_2O_3 measured in term of Absorbance at 600 nm against Al Concentration (ppm).

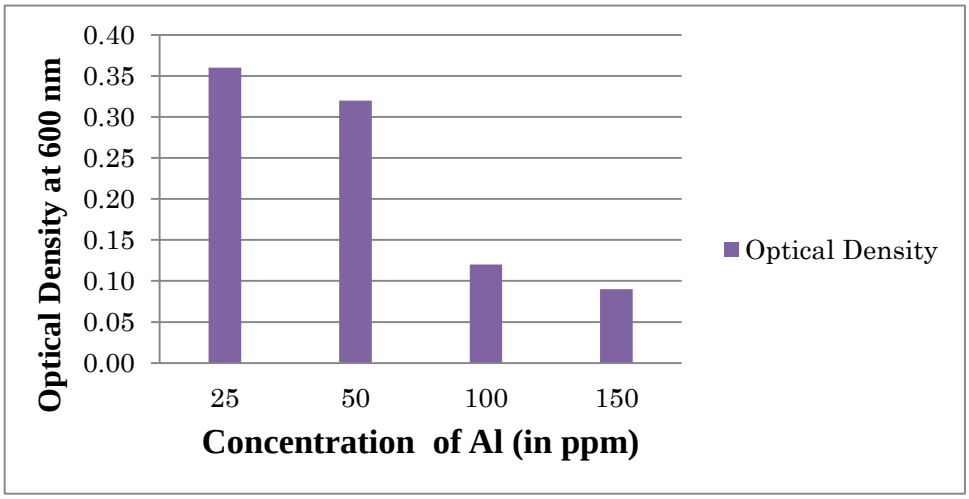

Fig No.4: Resistance of bacterial isolate -4 to Al_2O_3 measured in term of Absorbance at 600 nm against Al Concentration (PPM).

4. CONCLUSION

The bacterial resistance was studied with the treatment of varied concentrations of Aluminum. It was observed that, Isolate -3 showed highest tolerance in all Al_2O_3 concentrations (25 ppm, 50ppm, 100ppm, 150ppm) but showed highest tolerance in 100ppm concentration of Al_2O_3. . Isolate 1 showed highest tolerance in 50ppm concentration of Al_2O_3 . Isolate 4 showed highest tolerance in 25ppm concentration of Al_2O_3 . Isolate 2 showed highest concentration in 50ppm concentration of Al_2O_3 but showed weak growth with increased concentration. The highly aluminum resistant bacteria (Isolate-3) showed high resistant to antibiotic Cloxacilin and sensitive to Gentamicin, streptomycin and Rifampicin but more sensitive to Gentamicin.

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CONFLICT OF INTEREST

There is conflict of interest exists in completion this research work since it has been carried by the author as a partial fulfillment of M. Phil (Botany) course.

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