OBAFEMI AWOLOWO UNIVERSITY

ISOLATION, CHARACTERIZATION AND ANTIMICROBIAL EFFECT OF HUMIC ACID OBTAINED FROM SOIL AT DIFFERENT SITES IN ILE- IFE

BY

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CERTIFICATION

This is to certify that this research study was carried out by ADU Adedamola Abiodun (SCP11/12/H/0055) of the Institute of Ecology and Environmental Studies, as part of the requirement for the award of the degree of Master of Science (M.Sc.) in Environmental Control and Management, of the Obafemi Awolowo University, Ile-Ife, Nigeria.

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DEDICATION

This research work is dedicated to the Glory of The Almighty God and My late parents Mr. and Mrs. Adebola Victor Adu. May God grant them eternal rest.

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Words are not enough to qualify how much I appreciate my Boss, Mrs. Akinyemi O. A. You are a mother indeed in my life. Thank you ma for being there always; you will live to reap the fruits of your labor (Amen).

My profound appreciation goes to my family and friends, most especially my loving wife, Oluwabukunmi Adu; I am blessed to be part of you.

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ABSTRACT

This study characterized and determined the heavy metal load and antimicrobial activity of humic acid (HA) isolated from different soils in Ile– Ife. It also examined the plant anti- viral activity of the isolated HAs. This was with a view to assessing the prospect of the usage of humic acid as antimicrobial and plant growth agent.

Soil samples were collected from three different areas (hospital waste dumpsite of the Obafemi Awolowo University (OAU) Teaching Hospital, municipal waste dumpsite of Moremi Hall of Residence, OAU and an automobile workshop, where spent oil has been deposited overtime in Ile-Ife. Humic acid was extracted by treatment of the soil samples with dilute NaOH (1:10) in 250 ml conical flasks. The Infra Red Spectroscopy of isolated HA was determined using a Perkin Elmer Fourier Transform Infra Red (FT - IR) Spectrometer. The digests from soil samples were analyzed for the selected heavy metals (Pb, Cu, Cr and Fe) using Atomic Absorption Spectrophotometry. The antimicrobial activity of the isolates was tested against clinical isolates of some aerobic bacteria using agar well diffusion method. A standard antibiotic (streptomycin) was used as positive reference standard. Clinical isolates of the following microorganisms were used for the study: *Escherichia coli*, ***Proteus vulgaris***, *Klebsiella pneumoniae*, ***Bacillus polymyxa***, *Bacillus cereus*, ***Staphylococcus aureus***, *Clostridium sporogenes*, ***Corynebacterium pyogenes***, *Bacillus stearothermophilus*, ***Enterococcus faecalis***, *Micrococcus luteus*, ***Pseudomonas fluorescence*** and *Bacillus subtilis*. These microorganisms were obtained from the laboratory stock of the Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria. The antiviral activity of the isolates was evaluated using

two cowpea varieties (Ife Brown and TVu 76) as test plants in a Randomized Complete Block Design with six treatments which included the control experiment.

The results from the Fourier Transformed Infrared spectra of HA extracted from soils in Ile-Ife were found to be similar. The HA spectra showed strong hydrogen bonded O – H/ N – H stretching vibration at 3760.00 cm^{-1}, Dimeric O- H stretching vibration at 3453.00 cm^{-1}. A low intensity absorption indicative of an olefinic (C = C) or aromatic C = C bond was seen at 1639.57 cm^{-1}while an absorption at 1401.55 cm^{-1} indicative of phenols was also observed. Absorption at 1113.79 cm^{-1} and 1031.08 cm^{-1}indicative of C – O stretch of aliphatic or aromatic alcohol, cyclic/ acyclic esters, ethers or carboxylic acid. The HA from municipal waste dumpsite had less carbonyl content compared to that from hospital waste dumpsite and automobile workshop. The values of the heavy metals considered (Pb, Cu, Cr and Fe) for the soils and HAs from different sites are within the safe limit of the European commission except for Iron (Fe) with values higher than 1500 mg/kg which is the European commission limit. Results of the antimicrobial activity showed that HA displayed very good antimicrobial activity against *E. coli*, *P. vulgaris*, *K. pneumoniae*, *B. polymyxa*, *B. cereus*, *S. aureus*, *C. sporogenes*, *C. pyogenes*, *E. faecalis*, *P. fluorescence* and *B. subtilis* compared to the standard drug (streptomycin) but had no effect on *B. stearothermophilus* and *M. luteus*. For plant anti- viral activity, the treatments had significant effect on the yield parameters for the two cowpea varieties (Ife Brown and Tvu 76).

The study concluded that the isolated humic acids exhibited good broad spectrum antimicrobial activity and that humic acid was a multifunctional compound.

CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

Humic substances are major components of the natural organic matter in soil and water as well as in geological organic deposits such as lake sediments, peats, brown coals and shales. They make up much of the characteristic brown colour of decaying plant debris and contribute to the brown or black colour in surface soils. They are major components of Natural Organic matter in surface waters and at higher concentrations can impart a dark colour, especially in brown fresh water ponds, lakes and streams. In leaf litter or composts, the colour may be yellowish brown to black, depending on the degree of decay and concentration.

Humic substances are complex and heterogeneous mixtures of polydispersed materials formed by biochemical and chemical reactions during the decay and transformation of plant and microbial remains (a process called humification). Plant lignin, and its transformation products, as well as polysaccharides, melanin, cutin, proteins, lipids, nucleic acids, fine char particles, etc., are important components taking part in this process.

Numerous studies have shown that Humic Substances enhance root, leaf and shoot growth but also stimulate the germination of various crop species (Piccolo *et al.,* 1993). These positive effects are explained as an interaction between humic substance and physiological and metabolic processes (Musolo *et al.,* 1999; Nardi *et al.,* 2002). The addition of humic substance stimulate nutrient uptake (Dell'Agnola and Nardi, 1987; Piccolo *et al.,* 1993), cell permeability (Vaughan and Ord, 1981) and seems to regulate mechanisms involved in plant growth stimulation (Lee *et*

al., 1976; Dobbss *et al.,* 2007). It is not easy to distinguish between the direct and indirect effects of these substances. In fact, some of their positive effects may be ascribed to a general improvement of soil fertility, leading to a higher nutrient availability for plants. Whilst in other cases, humic substances seem to positively influence metabolic and signaling pathways involved in the plant development, by acting directly on specific physiological targets (Davis *et al.,* 2001; Danuta *et al.,* 2002). For this reason, understanding humic substance biological activity and the molecular mechanisms through which they exert their functions is becoming an important ecological task and a valid tool in facing environmental problems.

Humic substances in soils and sediments can be divided into three main fractions: humic acids (HA), fulvic acid (FA) and humin. These HA and FA are extracted from soil and other solid phase sources using strong base (NaOH or KOH). Humic acids are insoluble at low pH, and they are precipitated by adding strong acid. Humin cannot be extracted with either a strong base or a strong acid.

Humic substances are known to be of high molecular mass polyhydroxycarboxylates which contain poly-aromatic and aliphatic subunits. The degree of ionization of these macromolecules is governed by the amount of ionized phenolic and carboxylic groups, which is a function of pH of the solution. Their most common classification is based on the solubility at different pH values such as Humic acid , insoluble at pH less than 2 (under acidic condition); Fulvic acid, is soluble at all pH values and Humin, is insoluble at all pH values (Stevenson, 1972; Davies *et al.,* 2001; Karen *et al.,* 2005).

Humic acid is one of the most important forms of humic substances. Humic acid plays an important role in various fields such as agriculture, environment and biomedicine. On the other

hand, they can raise aquatic environment pollution, induce cytotoxicity for many mammalian cells and induce growth retardation and apoptosis of fibroblasts (Danuta *et al.,* 2002; Kuo-Jang *et al.,* 2003; Mei- Ling *et al.,* 2003; Thomas *et al.,* 2004). These important properties of humic acid have attracted attention of many investigators and the results over the years have bought new understanding on their structure and physicochemical properties.

Because humic acids are ubiquitous and can be extracted in different proportions by many solvents, they were extracted from many sources by varying methods. Naturally, the resulting extracts had different solubilities, colours, and textures, the principal properties used to differentiate compounds at the time.

1.2 Justification of the Study

Humic substances (consisting of humic acid, fulvic acid and humin) are ubiquitous in the environment. They exist on different waste media and in some instances constituting environmental nuisance. However, there is the prospect of the usage of humic acid as antimicrobial and plant growth agent when appropriately isolated, purified and applied, hence this study.

1.3 Objectives of the study

The objectives of the study are to isolate humic acid from different environment and determine the heavy metal load (Cr, Pb, Fe and Cu) in the soil samples and humic acid isolated from them. The specific objectives of the study are to

(a) characterize and quantify humic acid from the different sources;and
(b) determine the antimicrobial and plant anti- viral activities of the humic acid.

1.4 Contribution to Knowledge

This study has provided additional information on the antimicrobial and plant anti- viral activity of humic acid as well as its ability to increase the yield of cowpea.