

Production and Characterization of Humic Acid from Lignite and its Effects in Soil on Earthworms

Submitted in partial fulfillment of the requirements for the award of
Bachelor of Technology degree in Biotechnology

by

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DEPARTMENT OF BIOTECHNOLOGY

SCHOOL OF BIO AND CHEMICAL ENGINEERING

SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY

(DEEMED TO BE UNIVERSITY)

Accredited with Grade “A” by NNAC

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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **S Asaph (38230003)** and **Erin Jemimah Jayakirubai J (38230012)** who carried out the project entitled "**Production and Characterization of Humic Acid from Lignite and its Effects in Soil on Earthworms**" under my supervision from December 2021 to March 2022.


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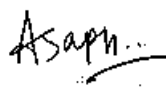
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DECLARATION

We **ASAPH.S (3823003)** and **ERIN JEMIMAH JAYAKIRUBAI J (38230012)** hereby declare that the Project Report entitled “**Production and Characterization of Humic Acid from Lignite and its Effects in Soil on Earthworms**” done by us under the guidance of **DR. PRAKASH P** is submitted in partial fulfilment of the requirements for the award of Bachelor of Technology degree in Biotechnology.

DATE: 21/04/2021

PLACE: CHENNAI

Handwritten signature of Asaph.S in black ink.Handwritten signature of Erin Jemimah Jayakirubai J in blue ink.

SIGNATURE OF THE CANDIDATE

ACKNOWLEDGEMENT

We are pleased to acknowledge our sincere thanks to the Board of Management of **SATHYABAMA** for their kind encouragement in doing this project and for completing it successfully. We are grateful to them.

We convey our thanks to **Dr. V. Ramesh Kumar, PhD, Dean, School of Bio and Chemical Engineering** and **Head of the Department, Dept. Of Biotechnology** for providing us necessary support and details at the right time during the progressive reviews.

We would like to express our sincere and deep sense of gratitude to our Project Guide **Dr. P. Prakash** for his valuable guidance, suggestions and constant encouragement paved way for the successful completion of my project work.

We wish to express our thanks to all Teaching and Non-teaching staff members of the Department of Biotechnology who were helpful in many ways for the completion of the project.

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ABSTRACT

Humic acids exist as a part of humus material, they are present abundantly in nature and form a natural pool of organic material. Lignite, as young coal, contains a significant amount of humic acid which can be used as an environmentally-friendly adsorbent for heavy metals. The real problem is that these heavy metals are almost always associated with humic acid. This is due to the high affinity of the humic acid for heavy metals and due to the presence of a number of functional radicals (hydroxyl, carboxyl, carbonyl, phenolic, and amine groups). Earthworms are used to study these ecotoxicological parameters as they are the primary source of contact with anything that affects soil fertility. Ecotoxicity studies are done in order to understand the effects of humic acid on earthworms. Bioaccumulation studies are carried out to estimate the amount of humic acid accumulated in the earthworms.

Keywords: Humic acid, Heavy metals, Ecotoxicity, Earthworms, Bioaccumulation

LIST OF ABBREVIATIONS

mg - Milligram

kg - Kilogram

% - Percentage

μS – Micro second

mS – Milli second

KOH - Potassium hydroxide

HA - Humic acid

SEM - Scanning Electron Microscope

EDS - Energy Dispersive Spectroscopy

ATR - IR - Attenuated Total Reflection - Infra-Red

ICP-OES - Inductively Coupled Plasma Optical Emission Spectrometry

BDL - Below Detection Limit

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CHAPTER 1: INTRODUCTION

1.1. LIGNITE

Coals are fossil fuels and they are said to be of four types: anthracite, bituminous, sub-bituminous and lignite. Out of these, sub-bituminous coal and lignite are considered lower ranking coals [40].

Lignite is a fossil fuel that is said to be produced in the early stages of coalification, and is also referred to as brown coal. It is a soft brown combustible sedimentary rock and it is formed from peat that has been naturally compressed. It is used as fuel for steam-electric power generation extensively. Lignite deposits are geologically young and therefore may contain plant debris. It makes up about 45% of the world's total coal reserves [7, 15, 29].

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Fig 1.1: Lignite (0.3 Micron)

1.1.1 CHARACTERISTIC FEATURES OF LIGNITE

Lignite is a non-agglomerating coal that is brownish-black in colour. It has a carbon content of roughly 25–35% and a gross calorific value of less than 4165 kcal/kg. The volatile matter in lignite is greater than 31% on a dry mineral matter-free basis. Lignite has a high moisture content in general and it can be as high as 66%. The ash content

ranges from 6% to 19%, whereas in bituminous coal it is 6–12%. Lignite has a relatively low heat content and this is why it is considered the lowest rank of coal. The heating value of lignite is less than 19.306 KJ/Kg [7, 15, 40].

1.2. HUMIC ACID

Humic acid is usually present in vegetable waste and can be detected as a black slime in the compost pit in a garden. It is also found in the brown organic matter of soil, peats, lignite, and brown coal. It is formed due to the bacterial and chemical degradation of plant tissue. In soils, it is likely to be formed by specific secondary processes such as “polymerization” of polyphenols leached by the rain, condensation of phenols, quinones & proteins which are provided by the action of soil micro-organisms and small animals on soil carbohydrates [5, 18, 20, 38].



Fig 1.2: Humic Acid

1.2.1. CHARACTERISTIC FEATURES OF HUMIC ACID

The characteristics of humic acid will vary depending on the origin of the soil and environment, but it is said to have certain common characteristics. The humic acid is a macromolecule and also a polyelectrolyte, its molecular weight ranges from 800 to 500,000. Humic acids have a base exchange capacity. These properties are considered important to soil stability, state of aggregation, transport of metal ions in the soil as well as through plant tissue. The fraction of humic substances that are not soluble in water under acidic conditions ($\text{pH} < 2$) is soluble at higher pH values. They can be separated from the soil by reagents that are insoluble in dilute acid. Humic

acids are the major extractable component of soil humic substances. Their colour ranges from dark brown to black [20, 38].

1.3. EARTHWORMS

Earthworms belong to the phylum Annelida and the Oligochaeta class. There are over 4400 different species of earthworms and they can be found in diverse habitats and feed upon various organic materials. They are primarily burrowing animals and feed on decaying organic matter, living bacteria, nematodes, rotifers, fungi, and other microbes [35].

Earthworms possess the ability to eat and mix large amounts of soil and organic matter and deposit them in the form of casts. They also enhance the incorporation and decomposition of organic matter, increase soil aggregate stability, improve porosity and water infiltration, and increase microbial activity.

Earthworms play an important role in the environment by cycling and releasing nutrients bound to detritus. They cooperate with microbes and speed up the process of decomposition. This, in turn, circulates nitrogen, carbon, phosphorus and improves the fertility of the soil [16].

Earthworms are divided into three main categories based on their ecological activity: epigeic, anecic, and endogeic earthworms.

1.3.1. *EUDRILUS EUGINIAE*

Eudrilus eugeniae is an anatomically advanced earthworm that has its origins in the tropical region of Western Africa, in the soils of the Savannah desert. The life cycle of this species from the stage of a cocoon to maturity is as short as 47 days [4].

The common name of the species *Eudrilus eugeniae* is African Nightcrawler and they are epigeic in nature. This means that they are inhabitants of the top layer of soil. Epigeic worms feed on the dead and decaying leaf litter and play important roles in both consumption and formation of humus [36].

1.3.2. CHARACTERISTIC FEATURES OF *EUDRILIUS EUGINIAE*

- Body length: Completely mature earthworms range around 90–185 mm or up to 250–400 mm under optimal conditions.
- Width: Ranges around 4–8 mm.
- Mass: 1.0 g or < 2g, under best conditions they can weigh about 5.0–6.0 g.
- Colour: Reddish-brown, ventrum beige, clitellum in a darker shade than the surroundings.
- Prostomium: Small, open epilobous.
- Dorsal pores: None.
- Setae: Eight per segment from 2 which are closely paired.
- Nephropores: Just behind the anterior furrow of each segment, longitudinal slits or slightly more median.
- Clitellum: around 13, 14, 15–18, commonly 13, 14–18.
- Male pores: In segment 17 on tips of longitudinally grooved, retracted as lateral slits with wrinkled lips just anterior to 17/18 in line with setae, eversible penes in large ventral chambers, tapering.
- Female pores: Combined with modified 'spermathecal pores' lateral, presetal in 14 as raised intrasegmental openings just anterior to setae.
- Genital markings: Centrally raised pad centered in 17 between male pores, very mildly repeated in 18, sometimes undeveloped or as elliptical, an opaque area in 16–18 [2, 13, 17].

1.3.3. FACTORS AFFECTING EARTHWORMS

- Natural/artificial environment

The type of environment they live in is the primary factor that helps study the survivability of any type of earthworm species [9, 10].

- Sunlight

It was observed that these species of earthworms were quite sensitive to the sun and preferred a darker environment.

- Temperature

The temperature of the soil must be suitable for the earthworms to survive.

- Water content

As important as water is to the soil and for the earthworms, it also determines the texture of the soil.

- Soil texture

The soil should not be too soggy, with clumps nor should it be too dry which will cause damage to the earthworms.

- Predators

Predators such as crows, rats, and ants can disturb their natural habitat.

- Migration

Migration from one place to another.

- Food

Vegetable and fruit waste must be supplied in adequate amounts, dumping too much waste at once can attract predators to invade their natural habitat.

- Oxygen level

Continuous and proper aeration is required in order for earthworms to survive. keeping it in a closed space will eventually result in its death.

- Heavy metal content

The amount of heavy metals present in the soil plays a major role [8, 14].

CHAPTER 2: LITERATURE SURVEY

Lignite is a naturally available substance that is present on the surface of the earth, its very own subsidiary humic acid is also found to be present in decaying fruits and vegetable waste. Humic acid is often used in the fields of industry, agriculture, medicine and environmental protection. A kind of potential organic resource that is being developed and utilized more and more, humic acid is seeking more attention from the world due to its properties. China is abundant in its coal resources and the lignite from China contains a lot of humic acid in them. Extraction of humic acid from these lignites creates favourable conditions for the improvement and development of coal and the agriculture industry and has a huge spectrum of utilization prospects. Humic acid can be extracted from lignite by using various modern day technology and techniques. The influence of nitrate concentrations, acid-coal proportions, oxidized temperature, oxidation time, extraction liquid concentration, liquid-solid ratio, extraction temperature and extraction time play a key role during its extraction process. Studies indicate that optimum processing conditions can be attained when sodium pyrophosphate and sodium hydroxide solutions are used as the extraction agents. The rate of humic acid production can reach 39.25% and the total content of humic acid production can be as high as 46.14%. Although being present naturally humic acid also has its own drawbacks like any other substance in this world [25]. Studies previously have shown that heavy metals such as copper, zinc, cobalt and cadmium have been associated closely with humic acid. The functional groups that are found predominantly in humic acid include alcohols, carboxylic acids, carbonyls, phenols, phosphates, sulfates, sulfides and amides all of which make humic acid optimal for metal interaction [23].

Earthworms are in direct contact with soil particles at all times, owing to their extremely permeable skin and digestive tract. As a result, they are naturally more impacted by toxins that are present in the soil system, this makes them ideal candidates for toxicity monitoring.

Findings from previous studies have demonstrated that earthworms have shown bioaccumulation tendencies toward heavy metals (Cd, Zn, Cu, Pb, etc.), nanoparticles and organic pollutants. Major physiological functions such as survival, nutrition, reproduction, immunity and growth are affected due to contaminants in the soil.

The reaction to pollution can be examined at various levels:

- a. whole-body level -weight loss, reduction in reproduction, tolerance or escape reaction, survivability
- b. tissue level - histopathological changes
- c. cellular level - changes in the physiology of the cells
- d. molecular level - expression levels of certain genes in response to the environmental stress [12, 32].

Bower (1979) noted that the extent of accumulation in biota depends on the chemical effects of the metal, its tendency to bind to particular materials and on the lipid content and composition of the biological tissue. Various activities by man in the present day has increased the quantity and distribution of heavy metals in land. The extent of this damage has raised concerns about the potential hazards these can have on plants, animals and humans. Soil heavy metal contamination is a very common problem in the terrestrial ecosystem that are ruined by industrial activities.

In China, it has been noted that almost 20% of the farmland soil has been contaminated by heavy metal accumulation. In addition to the concerns over heavy metal uptake by crop plants (bioaccumulation), a much better understanding of the potential for food chain transfer by earthworms and other soil fauna has become a very concerning topic. Numerous studies recently have examined the potential risks that the heavy metals can cause, and the evaluation of methods for the remediation is being tested regularly to ensure top notch food safety. However, few studies have been conducted to determine the exact relationship between heavy metals in the contaminated soils and different earthworm species inside the different ecological groups having different habitat and survivability strategies. Earthworms are considered to be important bio-indicators towards risk assessment and have been used in ecotoxicological studies to assess and understand the potential bioaccumulation and food chain transfer of heavy metals. Several earthworm species show “high tolerance” to heavy metal soil, suggesting differences related to different ecological groups or physiological tolerance to heavy metals. Different rate of bioaccumulation in various metals also appear to be related to differences in metal bio-availability as affected by environmental factors affecting solubility (pH and redox) and metal complexation with soil organic matter. In general, accumulation of metals

by earthworms occur through two pathways, which include absorption via dermal contact else by ingestion of organic matter and adsorption through the gut tissues. Due to the diversity of ecological characteristics and survival habits of different earthworms, the importance of dermal contact and gut adsorption may very obviously differ.

The earthworm species are generally classified into 3 broad ecological categories. They include epigeic earthworms that both feed and burrow in the litter layer on the surface of soils, endogeic earthworms that primarily inhabit the organic matter rich top soil profile, and anecic that move both vertically and horizontally in the soil and that form burrows in lower compartments of the soil profile. Due to small differences in heavy metal distributions in soil, it can be hypothesized that earthworms from different ecological groups will have completely different exposures to heavy metals and different potentials for metal bioaccumulation. With these differences in ecological conditions, it is of interest to determine the relationship between total soil of heavy metals and the internal metal concentrations in earthworm tissues, and to study the bioaccumulation factors for metals by different species of earthworm. Meanwhile, some field studies have been conducted to examine the bioaccumulation of metals by different earthworm species, differences in the uptake and accumulation of metals by multiple earthworm species in the very same soil have rarely been studied due to improper environmental conditions. Examination of the relationship between heavy metal concentrations and bioaccumulation was conducted (Hobbelenet al., 2006; Nahmani et al., 2009; Pérès et al., 2011; Lévêque et al., 2013; Nannoni et al., 2014), the study conducted here examined the different species and bioaccumulation levels of cadmium (Cd), zinc (Zn), copper (Cu) and lead (Pb) in soils having different levels of contamination [21].

CHAPTER 3: AIM AND SCOPE

3.1 AIM:

- To extract humic acid from lignite.
- To conduct characterization studies on the humic acid produced.
- To study the ecotoxicological effects of humic acid on *Eudrilulus euginae*.
- To study the bioaccumulation of humic acid in earthworms.

3.2 SCOPE:

- To find possible harmful effects on the morphology, behaviour and reproduction of the earthworm that are caused due to the heavy metals associated with humic acid.
- To find out the bioaccumulation rate of heavy metals in the earthworms.

CHAPTER 4: MATERIALS AND METHODS

4.1 MATERIALS:

- Lignite
- Potassium hydroxide
- Electronic balance
- Magnetic stirrer
- Centrifuge
- Sonicator
- Distilled water
- 15ml centrifuge tubes
- Ruler
- Red soil
- 13 inch - trays
- Spray bottles
- Metal mesh

4.2 PRODUCTION OF HUMIC ACID:

- 100 grams of lignite (0.3 microns) was taken in a beaker and mixed with 500 ml of distilled water.
- 4 grams of potassium hydroxide was added to it.
- A magnetic pellet was added to the beaker is placed on a magnetic stirrer and the stirrer was allowed to run for 4 hours.
- After this, the beaker was placed on an ultrasonicator and it is sonicated for 30 minutes.
- The slurry thus obtained was placed in a hot air oven at 60C overnight.
- Humic acid crystals were obtained.

4.3. CHARACTERIZATION STUDIES:

Humic Acid was given for Scanning Electron Microscopy (SEM) and Energy Dispersive Spectroscopy (EDS) analysis. This was done at the Sophisticated Analytical Instrument Facility (SAIF), IIT Madras.

The Lignite and Humic Acid were given for Attenuated Total Reflection - Infra-Red (ATR-IR) analysis at SAIF, IIT-M.

4.4. ECOTOXICITY STUDIES

Ecotoxicity tests are carried out at varying levels of biological organization, from the most precise levels i.e molecular level which causes biochemical changes to ecosystem function that is related to the environment. It can be modified for the direct measurements of soil toxicity. The general assays that are carried out at the organism level, constitute the single-species toxicity tests which can be applied only to that specific species. The final endpoints may either be lethal or sublethal effects that the particular substance can have on that specific organism (e.g., inhibition of growth or reproduction) [6, 11].

The ecotoxicity studies in this experiment were carried out over a period of 3 weeks.

18 trays that were 35 inches wide and 3 inches deep were filled with approximately 3.5 kg of soil each. Ten earthworms were added to each tray. There were 3 control trays (without the addition of any humic acid), and 15 trays with 5 different concentrations (0.001%, 0.025%, 0.5%, 0.75% and 0.01%) of humic acid. Triplicate concentrations were performed for the most accurate results. The trays were labeled appropriately. The humic acid was dissolved in 100 ml of water and spray bottles were used to spray the humic acid over 15 days.

4.4.1 MORPHOLOGY STUDIES

Morphology studies were conducted at intervals of 1 week. The length and mass of the earthworms have been recorded a total of 4 times over the course of the study. The overall appearance of the earthworms including signs of discoloration, swelling, and lesions in the body was monitored [36].

4.4.2 SURVIVAL STUDIES

The number of live earthworms was kept track of, in order to understand if there is mortality in the earthworms due to the subjection to humic acid. This was done periodically at the start of each week.

4.4.3 FECUNDITY STUDIES

Indications of earthworm fertility include cocoon production and hatchability. Observation was done for the appearance of juvenile earthworms and this was noted at regular intervals of time.

4.4.4 BEHAVIOURAL STUDIES

Behavioural studies were conducted on the earthworms during the course of the project. Any sort of sluggish behaviour or irregular movements was observed until the very last day.

4.4.5 BIOACCUMULATION

Bioaccumulation Factors (BAF) is calculated as the ratio of the elemental concentrations present in the earthworm to the total soil element content [22, 24]. Chemicals can accumulate in any type of organism by direct uptake from surrounding mediums such as water via gills, skin, etc., or by ingestion of particle-bound chemicals by the food chain leading to various pathways along with different trophic levels. These processes will not always occur with indirect adverse effects, like mortality, but also in complex phenomena, like reduced fertility, which leads to a risk for humans and the environment. It should be observed that even without traceable acute or chronic

effects in standard ecotoxicity tests bioaccumulation should be regarded as hazardous criteria, some effects can only be recognized in the later phase of life, which is called multi-generation effects, or can occur only in higher members of a food-web [19, 39]. Chemicals showing an indication of bioaccumulation potential, a bioaccumulation study might be necessary for the refinement of hazard assessment, depending on exposure situations and ecotoxicological data on a case-by-case decision. For chemicals showing no bioaccumulation potential, a bioaccumulation study does not have to be performed at this initial step of hazard assessment [23, 27, 31].

CHAPTER 5: RESULTS AND DISCUSSION

5.1 Characterization of Lignite:

5.1.1. ATR-IR analysis of lignite:

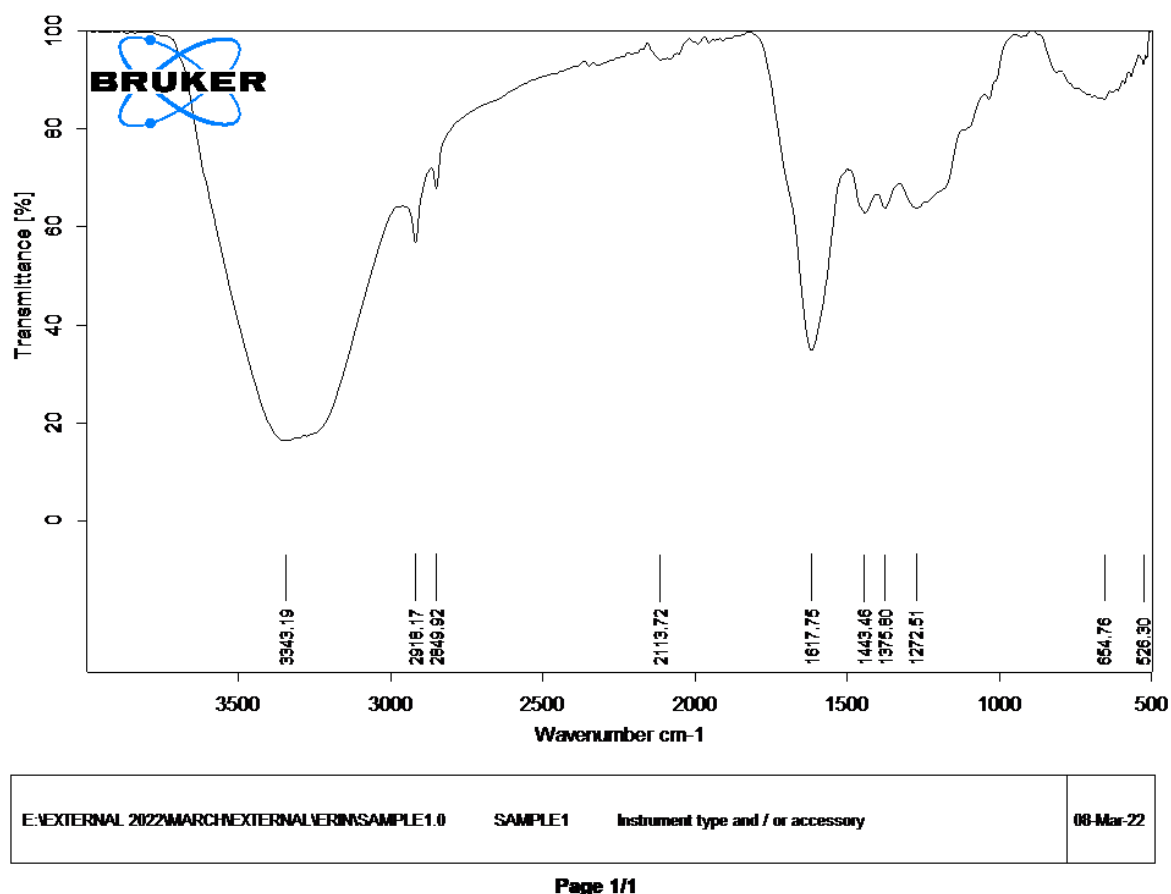
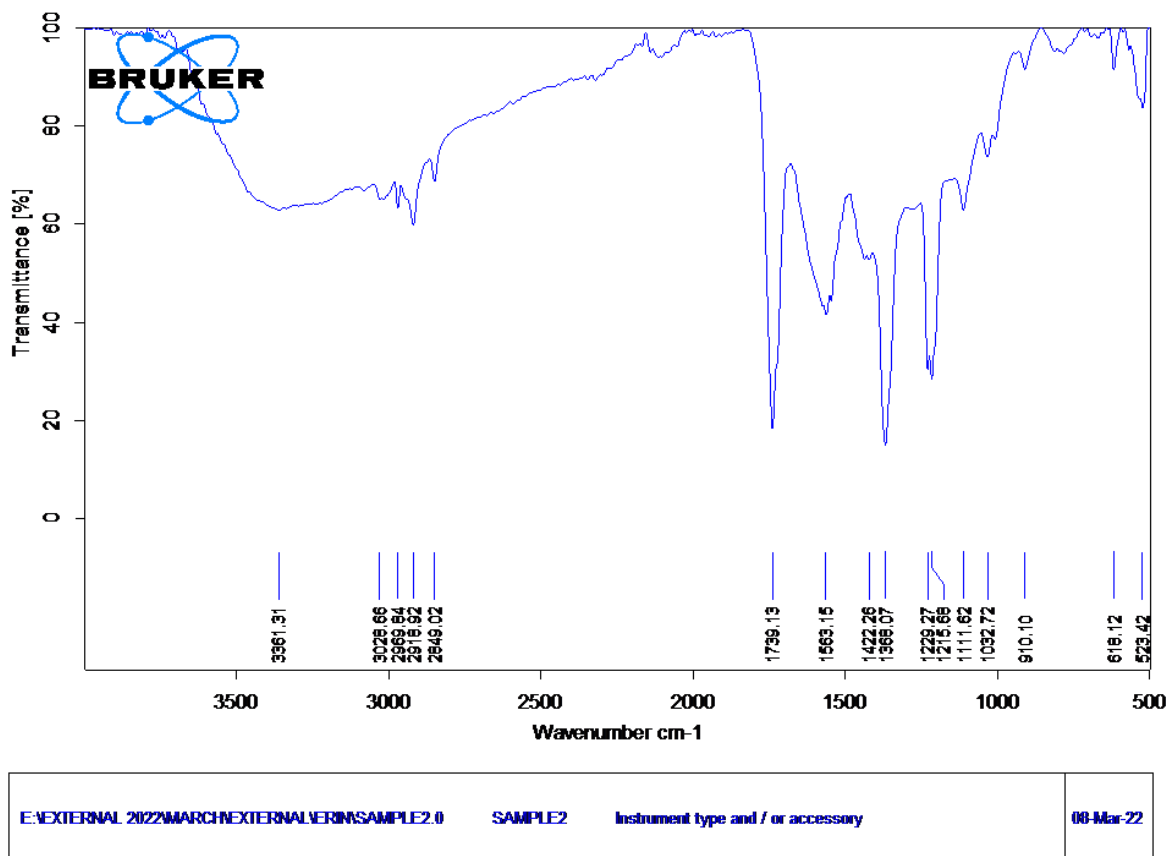


Fig 5.1: ATR-IR results for Lignite

The peak at 3343.19 indicates the presence of an O-H bond and the peak at 1617.75 indicates the presence of an -NH₂ bond.

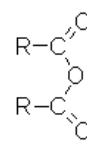
5.2 Characterization of Humic Acid:

5.2.1. ATR-IR analysis of HA:



Page 1/1

Fig. 5.2: ATR-IR results for Humic Acid



The peaks at 3361.31 and 1215.68 indicate the presence of a group and the peak at 1368.07 indicates the presence of an -O-CO-CH₃ group.

5.2.2. SEM analysis of Humic Acid:

The humic acid obtained from the lignite was given out for surface morphology analysis by the use of SEM. The analysis was carried out at SAIF, IIT Madras.

Fig 5.3- Fig 5.9 show that the analysis was done in 6 different magnifications for a clear understanding of the surface morphology of the given humic acid.

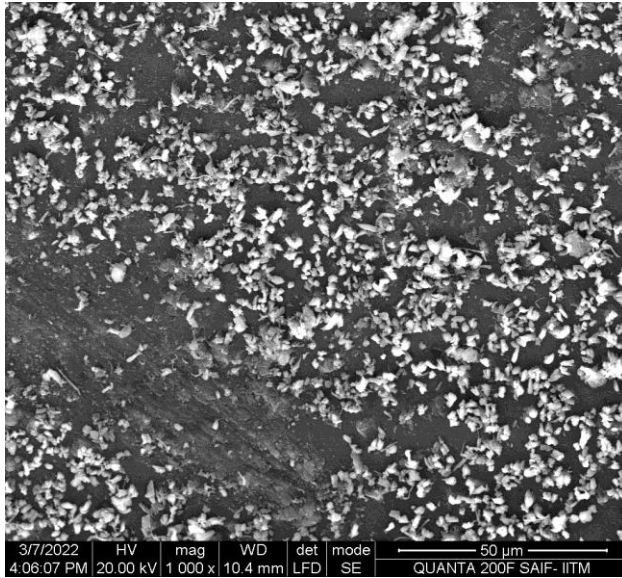


Fig 5.3: SEM image of HA at 1000 x magnification

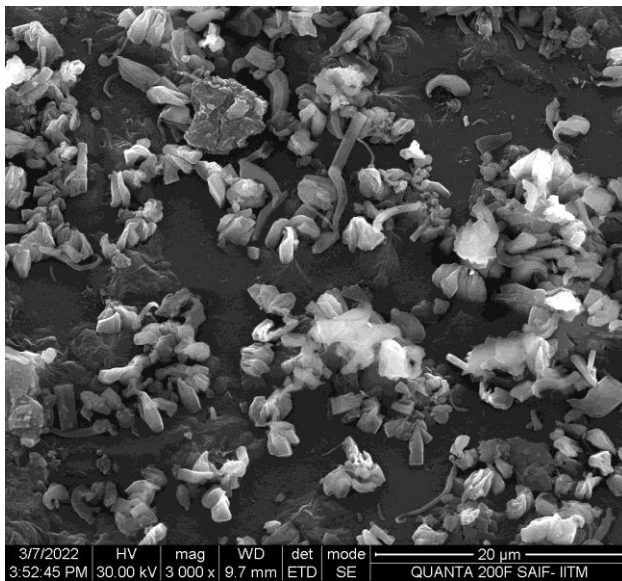


Fig 5.4: SEM image of HA at 3000 x magnification

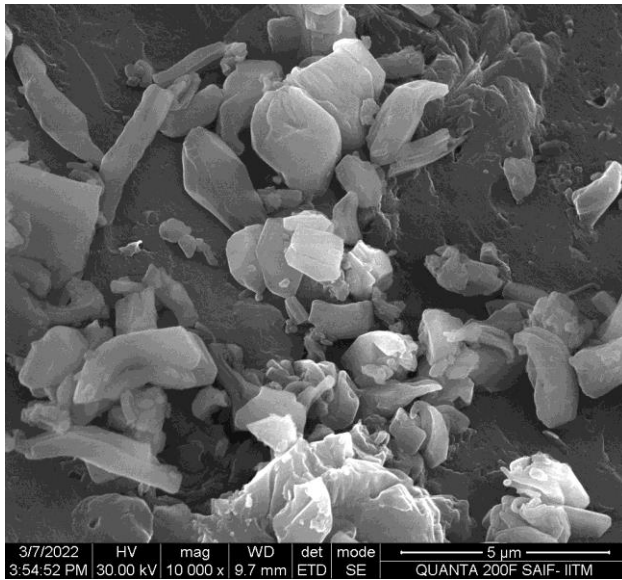


Fig 5.5: SEM image of HA at 10000 x magnification

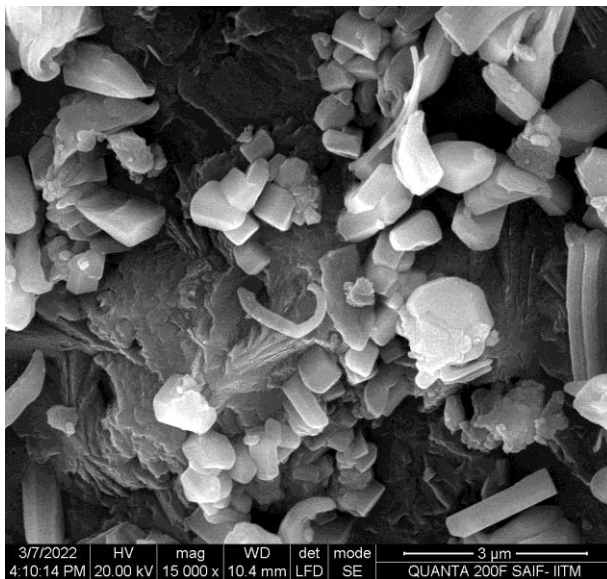


Fig 5.6: SEM image of HA at 15000 x magnification

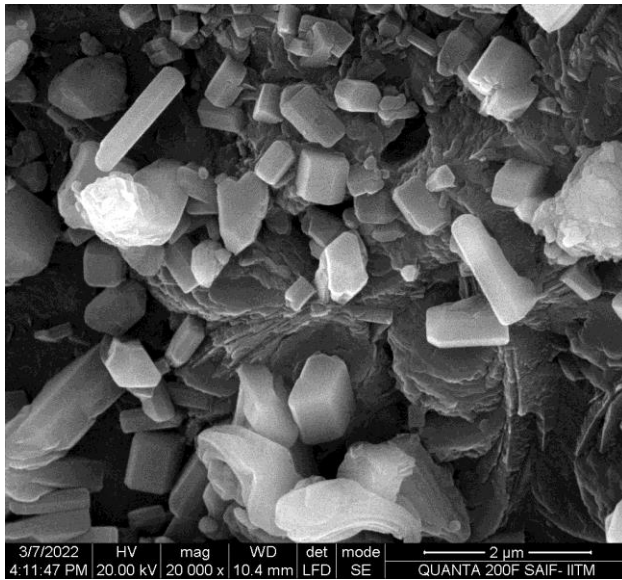


Fig 5.7: SEM image of HA at 20000 x magnification

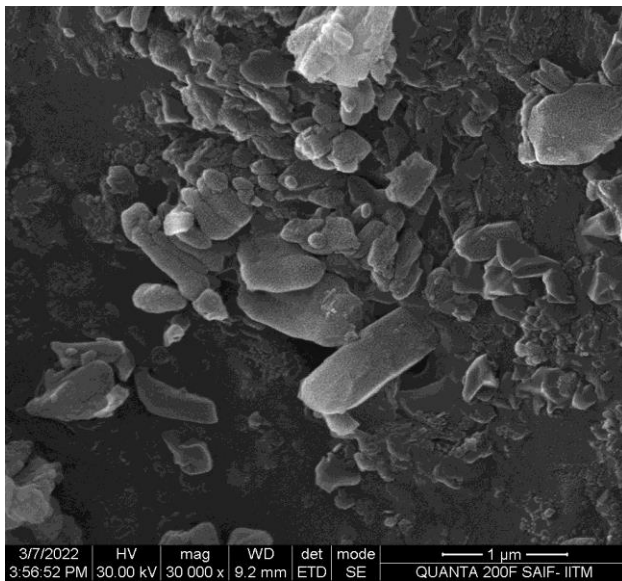


Fig 5.8: SEM image of HA at 30000 x magnification

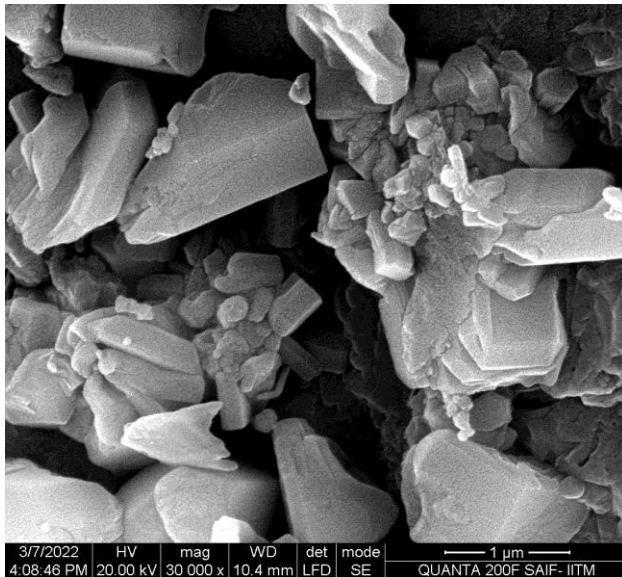


Fig 5.9: SEM image of HA at 30000 x magnification

5.2.3. EDS Analysis of Humic Acid:

c:\edax32\genesis\genmaps.spc 07-Mar-2022 16:12:37
LSecs : 35

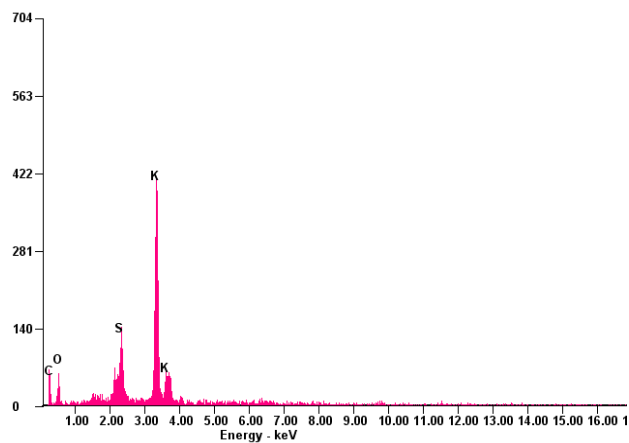


Fig 5.10: EDS Graph of HA

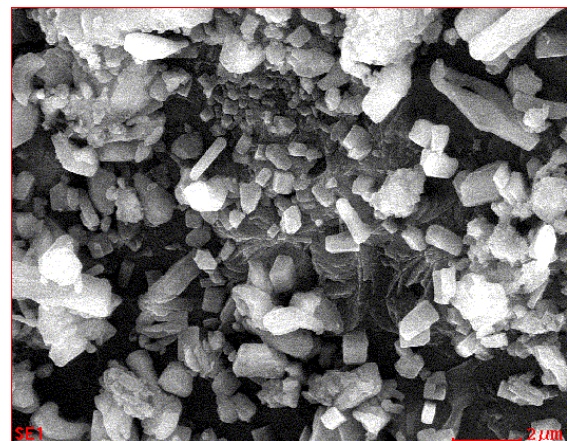


Fig 5.11: Spectrum 1

spectrum 1

TABLE: 5.1: EDS Results for HA spectrum 1

Element	Wt%	At%
CK	28.41	46.43
OK	23.02	28.24
SK	8.72	5.34
KK	39.84	20
Matrix	Correction	ZAF

The elemental analysis done using EDS indicates that elements such as carbon, oxygen, sulphur and potassium are present in spectrum 1.

c:\edax32\genesis\genmaps.spc 07-Mar-2022 16:15:15
LSecs : 30

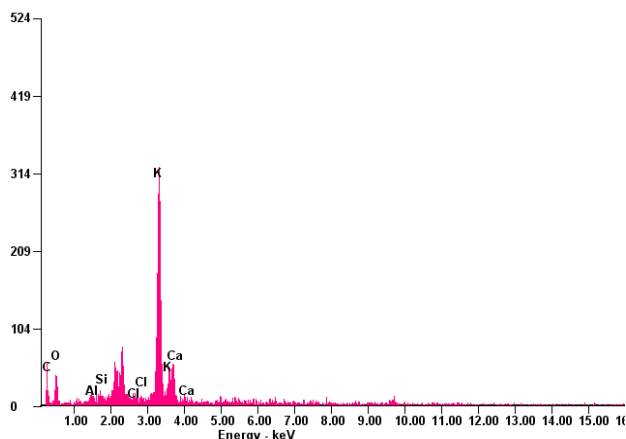


Fig 5.12: EDS Graph for HA spectrum 2

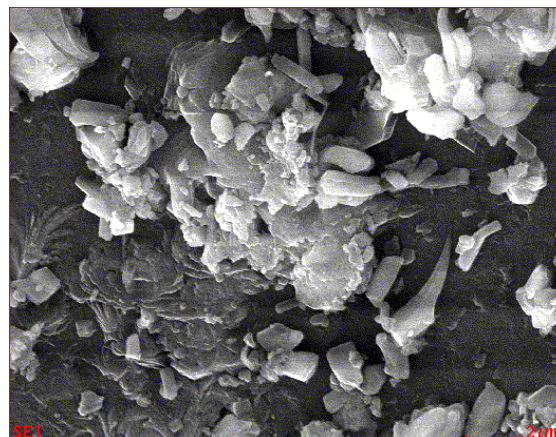


Fig 5.13: Spectrum 2

TABLE: 5.2: EDS Results for HA spectrum 2

Element	Wt%	At%
CK	35.05	50.97
OK	30.03	32.78
AlK	1.99	1.29
SiK	1.56	0.97
ClK	0.95	0.47
KK	24.85	11.1
CaK	5.56	2.42
Matrix	Correction	ZAF

In spectrum 2 it can be observed that elements such as carbon, oxygen, aluminium, silicon, chlorine, potassium and calcium are present in the Humic Acid.

5.3. Comprehensive Analysis of Humic Acid:

A comprehensive analysis of the humic acid produced was done at the National Agro Foundation, Taramani, Chennai.

The test method used was Biofertilizers and Organic Fertilizers in the Fertilizer (Control) Order 1985, Schedule IV – Part D, Schedule II – Part B - 3 (V)

TABLE 5.3: COMPREHENSIVE ANALYSIS OF HA

Parameter	Results	Unit
pH	6.40	-
Electrical conductivity	3200	$\mu\text{S}/\text{cm}$
Moisture	9.60	%
Total Nitrogen (Kjeldahl)	0.57	%
Total Phosphate as P_2O_5	8.80	%
Total Potash as K_2O	5.76	mg/Kg
Zinc as Zn	37.55	mg/Kg
Copper as Cu	21.23	mg/Kg
Lead as Pb	37.04	mg/Kg
Chromium as Cr	20.35	mg/Kg
Cadmium as Cd	3.59	mg/Kg
Nickel as Ni	46.42	mg/Kg
Total Organic Carbon	25.91	%
Particle size	92.0	%
Bulk density	0.80	g/cc
C:N ratio	45:1	-
Colour	Black	-

The results indicate that the pH of the humic acid is 6.40 and its electrical conductivity is 3200 $\mu\text{S}/\text{cm}$. The moisture content is 9.60%. Total Nitrogen, Phosphate and Potash are found in the percentages 0.57%, 8.80% and 5.76% respectively. 37.55 mg/Kg of Zinc, 21.23 mg/Kg of Copper, 37.04 mg/Kg of Lead, 20.35 mg/Kg of Chromium, 3.59 mg/Kg of Cadmium and 46.42 mg/Kg of Nickel are present in the humic acid. The total organic carbon was observed to be 25.91%. The Carbon to Nitrogen ratio was 45:1.

5.4. Soil Analysis:

Soil Analysis Studies was done at the National Agro Foundation, Taramani, Chennai.

The test method used was Methods of Analysis of Soils, Plants, Waters, Fertilisers and Organic Manures; Edited by H L S Tandon (FDCO), 2009 and Guide to Laboratory Establishment for Plant Nutrient Analysis by FAO UN, 2008.

TABLE 5.4: SOIL ANALYSIS

PARAMETER	RESULTS – Soil Sample 1	RESULTS – Soil Sample 2	UNIT
pH	4.88	6.80	-
Electrical Conductivity	0.097	0.256	mS/cm
Organic Matter	0.17	0.54	%
Nitrate (Nitrogen)	35.2	29.9	mg/kg
Available Phosphorus	3.95	9.03	mg/kg
Potassium (Exchangeable K)	95	296	mg/kg
Calcium (Exchangeable Ca)	503	576	mg/kg
Magnesium (Exchangeable Mg)	166	207	mg/kg
Sulfur (Available S)	15.6	53.0	mg/kg
Zinc (Available Zn)	0.33	0.66	mg/kg
Manganese (Available Mn)	16.78	29.72	mg/kg
Iron (Available Fe)	7.19	5.40	mg/kg
Copper (Available Cu)	1.03	1.07	mg/kg
Boron (Available B)	0.8	0.7	mg/kg
Sodium (Exchangeable Na)	310	459	mg/kg
Cation Exchange Capacity (by addition)	5.49	7.36	meq/100 g
K saturation	4.44	10.31	%
Ca Saturation	45.81	39.13	%
Mg Saturation	25.20	23.44	%
Na Saturation	24.55	27.12	%

Soil sample – 1 in the table is soil from the control trays and Soil Sample – 2 is soil that was subjected to 0.1% Humic Acid (Concentration – 5).

The above table is a comparison between soil samples 1 and 2. It clearly shows that the pH of the 2 samples are 4.88 and 6.80 respectively. This shows that soil sample 1 is more acidic in nature when compared to soil sample 2 which was subjected to the maximum concentration of humic acid. The electrical conductivity is 0.097 and 0.256 mS/cm. The presence of organic matter is 0.17% and 0.54%. The amount of Nitrogen was observed to be 35.2 mg/Kg and 29.9 mg/Kg. The available Phosphorus was 3.95 mg/Kg and 9.03 mg/Kg. The amount of exchangeable Potassium was 95 mg/Kg and 296 mg/Kg. The available Zinc content was 0.33 mg/Kg and 0.66 mg/Kg. The amount of Copper present was 1.03 mg/Kg and 1.07 mg/Kg. The amount of exchangeable Sodium was found to be 310 mg/Kg and 459 mg/Kg.

5.5. Morphology studies:

Table: 5.1 specifies the amount of humic acid that each of the trays were subjected to over the period of 15 days.

TABLE: 5.5: CONCENTRATIONS

Concentration	Percentage of HA	Mass of HA (in mg) per 3.5 kg of soil
Concentration 1	0.001%	35
Concentration 2	0.025%	875
Concentration 3	0.05%	1750
Concentration 4	0.075%	2625
Concentration 5	0.1%	3500

The first row in every table represents the length of earthworms measured on day 1 in that specific tray and the next row represents the mass of that particular earthworm.

The length was measured with the help of a regular ruler and the mass was measured using an electronic balance.



Fig 5.14: Weighing of Earthworm



Fig 5.15: Measuring of length

TABLE: 5.6: CONTROL TRAY-1

Control Tray 1	1	2	3	4	5	6	7	8	9	10	
Day 1	13	14	14	15	16	17	18	19	20	20	18.9
	0.51	0.61	0.71	0.75	0.8	0.92	0.93	0.95	0.97	0.99	0.931
Day 7	14	14	15	16	17	18	18	18	19	19	18.2
	0.71	0.7	0.77	0.72	0.94	0.96	0.98	0.94	1.41	1.39	1.173
Day 14	15	19	20	20	21	21	22	23	23	25	22.975
	0.77	1.4	1.14	1.11	1.06	1.42	1.52	1.38	1.24	1.7	1.3985
Day 21	17	19	20	21	21	21	22	23	24	26	23.6
	0.98	1.1	0.91	1.42	1.39	1.07	1.24	1.37	1.64	1.74	1.509

TABLE: 5.7: CONTROL TRAY-2

Control Tray 2	1	2	3	4	5	6	7	8	9	10	
Day 1	13	13	13	15	16	18	19	19	21	22	19.725
	0.64	0.63	0.64	0.74	0.71	0.95	0.98	0.98	0.94	1.24	1.00125
Day 7	16	19	19	19	19	20	21	21	22	22	21.2
	0.73	1.01	0.99	0.92	1.41	1.13	1.42	1.3	1.24	1.08	1.18575
Day 14	19	20	20	20	21	21	21	22	23	23	22.25
	1.41.	1.16	1.14	0.93	1.4	1.07	1.2	1.24	1.38	1.41	1.3111
Day 21	20	21	21	22	22	23	23	23	23	24	23.05
	1.38	1.41	0.94	1.29	1.52	1.23	1.41	1.38	1.27	1.65	1.412

TABLE: 5.8: CONTROL TRAY-3

Control Tray 3	1	2	3	4	5	6	7	8	9	10	
Day 1	13	14	15	16	16	17	17	18	18	19	17.825
	0.64	0.7	0.78	0.73	0.5	0.98	1	0.84	0.87	1	0.8785
Day 7	17	17	17	18	18	18	19	19	20	21	19.6
	1.01	0.97	0.89	0.86	0.85	0.91	0.91	0.95	0.93	1.07	0.97125
Day 14	20	20	20	20	21	21	22	23	23	25	23.125
	1.16	1.1	1.05	1.09	1.07	0.98	1.08	1.24	1.33	1.69	1.36527
Day 21	20	20	21	21	21	21	22	23	24	25	23.45
	1.12	0.91	0.94	1.08	1.4	1.33	1.52	1.37	1.52	1.68	1.46425

TABLE: 5.9: CONCENTRATION-1 TRAY-1

Conc Tray 1	1	2	3	4	5	6	7	8	9	10	
Day 1	13	15	15	15	16	16	17	17	18	18	17.25
	0.65	0.76	0.78	0.77	0.75	0.74	0.9	1.08	0.9	0.84	0.90925
Day 7	15	19	19	20	20	20	20	21	21	22	20.925
	0.77	1	0.93	0.95	0.91	0.98	1	1.07	1	1.08	1.02975
Day 14	18	20	20	21	21	21	21	22	22	22	21.7
	0.91	1	0.97	1.11	1.08	1.16	1.04	1.2	1.17	1	1.10916
Day 21	19	20	21	21	21	22	22	23	23	23	22.625
	0.97	1.16	1.4	1.42	1.33	1.37	1.31	1.27	1.38	1.41	1.3405

TABLE: 5.10: CONCENTRATION-1 TRAY-2

Conc Tray 2	1	2	3	4	5	6	7	8	9	10	
Day 1	15	16	17	17	17	17	18	18	18	19	18.05
	0.78	0.73	1	0.99	0.96	0.98	0.89	0.9	0.94	0.91	0.9145
Day 7	16	18	18	19	19	20	20	21	21	22	20.85
	0.77	0.93	0.91	0.93	0.95	0.98	0.91	1.09	1.04	1.08	1.04225
Day 14	17	21	21	22	22	22	23	23	23	23	22.675
	1.02	1.07	0.94	1.2	1.14	1.17	1.2	1.14	1.31	1.38	1.24675
Day 21	18	21	22	22	23	23	23	24	24	24	23.6
	0.84	1.2	1.24	1.22	1.31	1.38	1.33	1.49	1.51	1.41	1.42575

TABLE: 5.11: CONCENTRATION-1 TRAY-3

Conc Tray 3	1	2	3	4	5	6	7	8	9	10	
Day 1	13	14	15	15	16	17	18	19	19	19	18.375
	0.67	0.73	0.77	0.78	0.76	1.05	0.87	0.91	0.94	1.01	0.92725
Day 7	15	17	18	18	18	18	19	20	21	22	20.4
	0.79	0.96	0.87	0.9	0.9	0.83	0.95	0.91	1.07	1.2	1.0295
Day 14	17	19	20	20	21	22	22	23	24	25	23.325
	0.97	0.98	1.07	1.02	1.1	1.08	1.12	1.27	1.4	1.68	1.37975
Day 21	20	21	21	21	22	22	23	23	25	25	23.825
	1.16	1.33	1.42	1.31	1.24	1.33	1.41	1.62	1.67	1.69	1.5995

TABLE: 5.12: CONCENTRATION-2 TRAY-1

Conc Tray 1	1	2	3	4	5	6	7	8	9	10	
Day 1	13	13	15	15	15	16	18	18	20	22	19.125
	0.64	0.63	0.76	0.79	0.76	0.78	0.89	0.95	0.94	0.93	0.90675
Day 7	16	17	18	19	19	20	20	20	20	23	20.55
	0.79	0.98	0.89	0.97	0.91	0.94	1.16	1.1	1.08	1.26	1.112
Day 14	20	21	22	24	24	24	25	25	25	25	24.625
	1.17	1.07	1.21	1.44	1.37	1.41	1.52	1.63	1.7	1.68	1.6075
Day 21	21	21	24	24	24	25	25	25	25	26	25
	1.42	1.41	1.47	1.44	1.52	1.64	1.61	1.6	1.69	1.73	1.64325

TABLE: 5.13: CONCENTRATION-2 TRAY-2

Conc 2 Tray 2	1	2	3	4	5	6	7	8	9	10	
Day 1	13	14	14	16	16	18	18	18	19	19	18.125
	0.61	0.71	0.7	0.8	0.78	0.85	0.83	0.87	0.97	0.96	0.902
Day 7	18	18	19	20	20	21	23	24	25	25	23.825
	0.95	0.98	0.98	1.17	1.13	0.94	1.14	1.08	1.22	1.2	1.14475
Day 14	20	21	21	21	21	22	23	24	25	25	24.075
	1.16	0.99	0.95	1.1	1.07	1.24	1.38	1.41	1.47	1.5	1.40175
Day 21	21	21	21	21	22	23	23	25	25	25	24.425
	1.42	1.41	1.38	1.33	1.22	1.21	1.35	1.69	1.7	1.57	1.597

TABLE: 5.14: CONCENTRATION-2 TRAY-3

Conc 2 Tray 3	1	2	3	4	5	6	7	8	9	10	
Day 1	14	15	16	16	16	17	18	19	19	19	18.475
	0.72	0.79	0.77	0.77	0.8	1	0.96	0.97	0.89	0.95	0.918
Day 7	14	19	19	20	20	20	21	21	22	24	21.75
	0.7	0.93	0.91	0.94	0.98	1.05	0.99	1.01	1.08	1.32	1.10025
Day 14	14	20	21	21	21	22	22	22	22	25	22.5
	0.71	0.94	1.1	1.03	0.99	1.24	1.2	1.18	1.21	1.47	1.24175
Day 21	16	20	21	21	22	22	23	23	24	25	23.425
	0.73	1.21	1.42	1.37	1.51	1.49	1.38	1.24	1.69	1.71	1.50375

TABLE: 5.15: CONCENTRATION-3 TRAY-1

Conc 3 Tray 1	1	2	3	4	5	6	7	8	9	10	
Day 1	14	14	15	15	16	16	17	17	17	22	18.075
	0.72	0.71	0.77	0.77	0.73	0.77	0.96	1.03	0.97	1.08	0.98275
Day 7	14	15	15	16	18	18	18	19	21	22	19.9
	0.74	0.76	0.79	0.81	1	0.98	0.96	0.93	0.94	1.02	0.94575
Day 14	16	20	20	20	21	21	21	22	22	23	21.9
	0.75	0.94	0.91	1	0.94	0.98	1.03	1.08	1.16	1.26	1.12625
Day 21	18	20	20	20	20	21	21	22	22	23	21.925
	0.94	1.16	1.2	1.21	0.94	1.4	1.39	1.52	1.47	1.69	1.49

TABLE: 5.16: CONCENTRATION-3 TRAY-2

Conc 3 Tray 2	1	2	3	4	5	6	7	8	9	10	
Day 1	14	14	14	15	16	16	17	17	19	19	17.775
	0.7	0.74	0.73	0.77	0.76	0.75	0.82	0.87	0.98	0.94	0.899
Day 7	14	15	16	16	16	18	18	19	21	21	19.6
	0.69	0.78	0.77	0.79	0.78	0.91	0.89	1.06	1.07	1.02	1.0065
Day 14	15	15	17	17	18	18	18	19	21	23	20.275
	0.78	0.79	0.89	0.92	0.93	1.04	0.98	0.91	1.07	1.29	1.0575
Day 21	17	17	17	18	18	18	18	19	21	23	20.4
	0.94	0.96	0.92	1.02	0.99	1.02	0.95	0.95	1.37	1.41	1.19575

TABLE: 5.17: CONCENTRATION-3 TRAY-3

Conc Tray 3	3	1	2	3	4	5	6	7	8	9	10	
Day 1		14	14	14	14	15	16	18	19	19	21	18.85
		0.75	0.77	0.74	0.72	0.77	0.73	1.01	0.95	0.91	0.94	0.90725
Day 7		14	14	15	15	17	17	19	19	20	22	19.55
		0.71	0.73	0.79	0.77	1.1	0.89	0.9	0.91	0.92	0.94	0.909
Day 14		15	16	17	18	18	18	19	20	22	24	21.175
		0.78	0.76	0.88	0.96	0.89	0.85	0.97	1.05	1.06	1.21	1.06525
Day 21		15	17	17	18	19	19	20	20	23	24	21.55
		0.79	0.83	0.86	0.9	0.93	0.98	1.19	1.17	1.2	1.52	1.23175

TABLE: 5.18: CONCENTRATION-4 TRAY-1

Conc Tray 1	4	1	2	3	4	5	6	7	8	9	10	
Day 1		13	16	17	17	17	18	19	19	19	22	19.425
		0.65	0.74	0.88	0.93	0.81	0.91	0.89	0.94	0.9	0.99	0.9235
Day 7		16	17	17	18	18	19	19	20	21	22	20.425
		0.75	0.84	0.95	0.94	0.93	1.02	0.98	0.1	1.07	1.13	0.79275
Day 14		19	19	19	20	20	20	21	21	21	23	21.325
		1.04	1.23	1.4	0.91	1.03	0.96	1.12	0.94	1.04	1.24	1.07775
Day 21		19	19	20	20	21	21	21	23	23	23	22.5
		1.42	1.15	0.98	1.09	1.07	1.32	1.4	1.33	1.41	1.38	1.34375

TABLE: 5.19: CONCENTRATION-4 TRAY-2

Conc Tray 2	4	1	2	3	4	5	6	7	8	9	10	
Day 1		13	13	14	14	14	15	16	16	18	20	17.325
		0.65	0.63	0.77	0.72	0.74	0.76	0.72	0.74	0.96	0.97	0.859
Day 7		13	15	15	16	17	17	18	19	19	23	19.55
		0.67	0.78	0.79	0.77	0.92	0.81	0.94	1.39	0.91	1.03	1.05775
Day 14		14	17	17	17	18	18	19	20	20	26	21.15
		0.71	0.81	1	0.86	1	0.103	1.38	0.95	1.16	1.47	1.13107
Day 21		15	17	17	18	18	19	21	22	23	26	22.65
		0.77	0.88	0.9	1.03	0.97	1.41	1.42	1.51	1.38	1.73	1.455

TABLE: 5.20: CONCENTRATION-4 TRAY-3

Conc Tray 3	4	1	2	3	4	5	6	7	8	9	10	
Day 1		14	15	15	16	17	18	18	19	19	21	19.05
		0.74	0.79	0.79	0.75	0.82	0.85	0.87	1.33	1.39	1.02	1.16875
Day 7		16	15	16	19	19	19	20	21	21	22	20.7
		0.77	0.77	0.8	0.92	1.12	1.3	1.16	0.99	1.08	1.14	1.05375
Day 14		16	16	18	20	20	20	21	22	22	23	21.7
		0.75	0.72	0.84	0.97	0.91	0.95	1.07	1.24	1.17	1.2	1.148
Day 21		17	17	18	20	21	22	22	22	23	23	22.125
		0.84	0.87	0.91	1.17	1.1	1.52	1.24	1.31	1.38	1.43	1.32425

TABLE: 5.21: CONCENTRATION-5 TRAY-1

Conc Tray 1	5	1	2	3	4	5	6	7	8	9	10	
Day 1		13	14	14	15	16	17	17	18	18	19	17.775
		0.64	0.75	0.77	0.79	0.76	0.85	0.88	1	1.02	1	0.9665
Day 7		16	16	17	17	18	18	19	20	21	21	20.075
		0.78	0.78	0.9	0.96	1.04	1	1.07	0.98	0.94	1.01	0.969
Day 14		18	18	18	19	19	20	20	20	21	23	20.9
		0.95	0.84	0.98	1.15	1.01	1.16	1.18	1.06	1.07	1.24	1.1085
Day 21		18	19	19	20	21	21	21	22	23	23	22.175
		0.99	1.23	1.29	1.15	1.4	1.37	1.31	1.52	1.38	1.24	1.357

TABLE: 5.22: CONCENTRATION-5 TRAY-2

Conc Tray 2	5	1	2	3	4	5	6	7	8	9	10	
Day 1		15	15	15	15	15	16	16	17	17	18	16.975
		0.78	0.79	0.78	0.77	0.78	0.74	0.72	0.83	0.81	0.89	0.82975
Day 7		16	16	17	18	19	19	20	20	21	21	20.175
		0.75	0.78	0.87	1	0.98	0.97	0.91	0.93	1.06	0.98	0.97325
Day 14		18	19	20	20	21	21	21	22	22	23	21.925
		1.06	1.1	0.92	0.99	0.97	1.05	0.96	1.08	1.24	1.29	1.169
Day 21		18	19	21	21	21	22	22	23	23	23	22.575
		0.97	1.37	1.41	1.33	1.14	1.52	1.34	1.38	1.24	1.39	1.32975

TABLE: 5.23: CONCENTRATION-5 TRAY-3

Conc 5 Tray 3	1	2	3	4	5	6	7	8	9	10	
Day 1	16	16	16	16	17	18	18	19	20	20	19.15
	0.73	0.75	0.73	0.72	0.88	0.84	0.89	1.02	0.92	0.9	0.9195
Day 7	16	17	17	18	19	19	20	20	21	23	20.75
	0.77	0.91	0.87	0.92	1.22	1.08	0.98	1.01	0.99	1.23	1.057
Day 14	17	17	19	19	19	20	20	21	21	24	21.425
	1.03	0.82	1.01	1.2	1.09	1.16	1.06	1.42	1.41	1.41	1.35025
Day 21	17	18	19	20	21	21	22	23	23	24	22.7
	0.98	1.06	1.41	1.17	1.38	1.45	1.56	1.41	1.39	1.68	1.45725

TABLE: 5.6- TABLE: 5.23 show that the length and mass were recorded every week until the 21st day. The average length and mass were calculated for the 1st, 7th, 14th, and 21st days. It is evident that all the 18 trays had similar growth that differed by just a few decimal places, proving that the humic acid did not inhibit their growth. There were no abnormalities in the appearance of any earthworm. All the earthworms appeared healthy.

5.6 SPSS:

The acronym SPSS originally stands for “Statistical Package for the Social Sciences” (SPSS). Later the original market changed it to “Statistical Product and Service Solutions”. SPSS is a widely used specialized tool for statistical analysis in social science. It is often used by market researchers, health researchers, survey companies, government, education researchers, marketing organizations, data miners etc [1].

5.6.1. LENGTH STATISTICS:

Paired Samples t-test for Day 7

Pair 1:

H₀1: There is no significant difference between the means of Length of Earthworm on Without Humic Acid and Humic Acid 100 mg for Day 7.

H₁1: There is significant difference between the means of Length of Earthworm on Without Humic Acid and Humic Acid 100 mg for Day 7.

Pair 2:

H₀1: There is no significant difference between the means of Length of Earthworm on Without Humic Acid and Humic Acid 250 mg for Day 7.

H₁1: There is significant difference between the means of Length of Earthworm on Without Humic Acid and Humic Acid 250 mg for Day 7.

Pair 3:

H₀1: There is no significant difference between the means of Length of Earthworm on Without Humic Acid and Humic Acid 500 mg for Day 7.

H₁1: There is significant difference between the means of Length of Earthworm on Without Humic Acid and Humic Acid 500 mg for Day 7.

Pair 4:

H₀1: There is no significant difference between the means of Length of Earthworm on Without Humic Acid and Humic Acid 750 mg for Day 7.

H₁1: There is significant difference between the means of Length of Earthworm on Without Humic Acid and Humic Acid 750 mg for Day 7.

Pair 5:

H₀1: There is no significant difference between the means of Length of Earthworm on Without Humic Acid and Humic Acid 1000 mg for Day 7.

H₁1: There is significant difference between the means of Length of Earthworm on Without Humic Acid and Humic Acid 1000 mg for Day 7.

TABLE: 5.24**Paired Samples Statistics**

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	NOHD7	18.4	10	1.506	0.476
	H100D7	19.2	10	1.989	0.629
Pair 2	NOHD7	18.4	10	1.506	0.476
	H250D7	20.2	10	2.251	0.712
Pair 3	NOHD7	18.4	10	1.506	0.476
	H500D7	17.5	10	2.635	0.833
Pair 4	NOHD7	18.4	10	1.506	0.476
	H750D7	18.2	10	2.15	0.68
Pair 5	NOHD7	18.4	10	1.506	0.476
	H1000D7	18.8	10	2.044	0.646

TABLE:5.25**Paired Samples Correlations**

		N	Correlation	Sig.
Pair 1	NOHD7 & H100D7	10	0.935	0
Pair 2	NOHD7 & H250D7	10	0.957	0
Pair 3	NOHD7 & H500D7	10	0.98	0
Pair 4	NOHD7 & H750D7	10	0.968	0
Pair 5	NOHD7 & H1000D7	10	0.968	0

TABLE: 5.26**Paired Samples Test**

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	NOHD7 - H100D7	-0.8	0.789	0.249	-1.364	-0.236	-3.207	9	0.011
Pair 2	NOHD7 - H250D7	-1.8	0.919	0.291	-2.457	-1.143	-6.194	9	0
Pair 3	NOHD7 - H500D7	0.9	1.197	0.379	0.044	1.756	2.377	9	0.041
Pair 4	NOHD7 - H750D7	0.2	0.789	0.249	-0.364	0.764	0.802	9	0.443
Pair 5	NOHD7 - H1000D7	-0.4	0.699	0.221	-0.9	0.1	-1.809	9	0.104

Inference

Pair 1:

To test the hypothesis that Without Humic Acid (Mean = 18.40, Standard Deviation = 1.506) and Humic Acid means (Mean = 19.20, Standard Deviation = 1.989) were equal, paired sample t-test has been performed. The correlation value $r = .935$, $p = .009$, there exists a positive correlation between the variables. Since the sig value is 0.000, which is < 0.05 , reject null hypothesis. There is a significant difference between the means of Length of Earthworm on Without Humic Acid and Humic Acid 100 mg for Day 7.

Pair 2:

To test the hypothesis that Without Humic Acid (Mean = 18.40, Standard Deviation = 1.506) and Humic Acid means (Mean = 20.20, Standard Deviation = 2.251) were equal, paired sample t-test has been performed. The correlation value $r = .957$, $p = .003$, there exists a perfect positive correlation between the variables. Since the sig value is 0.000, which is < 0.05 , reject null hypothesis. There is a significant difference between the means of Length of Earthworm on Without Humic Acid and Humic Acid 250 mg for Day 7.

Pair 3:

To test the hypothesis that No Humic Acid (Mean = 18.40, Standard Deviation = 1.506) and Humic Acid means (Mean = 17.50, Standard Deviation = 2.635) were equal, paired sample t-test has been performed. The correlation value $r = .980$, $p = .000$, there exists a perfect positive correlation between the variables. Since the sig value is 0.000, which is < 0.01 , reject null hypothesis. There is a significant difference between the means of Length of Earthworm on Without Humic Acid and Humic Acid 500 mg for Day 7.

Pair 4:

To test the hypothesis that Without Humic Acid (Mean = 18.40, Standard Deviation = 1.506) and Humic Acid means (Mean = 18.20, Standard Deviation = 2.150) were equal, paired sample t-test has been performed. The correlation value $r = .968$, $p = .005$, there exists a perfect positive correlation between the variables. Since the sig value is .000, which is < 0.05 , reject null hypothesis. There is a significant difference

between the means of Length of Earthworm on Without Humic Acid and Humic Acid 750 mg for Day 7.

Pair 5:

To test the hypothesis that Without Humic Acid (Mean = 18.40, Standard Deviation = 1.506) and Humic Acid means (Mean = 18.80, Standard Deviation = 2.044) were equal, paired sample t-test has been performed. The correlation value $r = .968$, $p = .010$, there exists a positive correlation between the variables. Since the sig value is 0.000, which is < 0.05 , reject null hypothesis. There is a significant difference between the means of Length of Earthworm on Without Humic Acid and Humic Acid 1000 mg for Day 7.

Paired Samples t-test for Day 14

Pair 1:

H₀1: There is no significant difference between the means of Length of Earthworm on Without Humic Acid and Humic Acid 100 mg for Day 14.

H₁1: There is significant difference between the means of Length of Earthworm on Without Humic Acid and Humic Acid 100 mg for Day 14.

Pair 2:

H₀1: There is no significant difference between the means of Length of Earthworm on Without Humic Acid and Humic Acid 250 mg for Day 14.

H₁1: There is significant difference between the means of Length of Earthworm on Without Humic Acid and Humic Acid 250 mg for Day 14.

Pair 3:

H₀1: There is no significant difference between the means of Length of Earthworm on Without Humic Acid and Humic Acid 500 mg for Day 14.

H₁1: There is significant difference between the means of Length of Earthworm on Without Humic Acid and Humic Acid 500 mg for Day 14.

Pair 4:

H₀1: There is no significant difference between the means of Length of Earthworm on Without Humic Acid and Humic Acid 750 mg for Day 14.

H₁1: There is significant difference between the means of Length of Earthworm on Without Humic Acid and Humic Acid 750 mg for Day 14.

Pair 5:

H₀1: There is no significant difference between the means of Length of Earthworm on Without Humic Acid and Humic Acid 1000 mg for Day 14.

H₁1: There is significant difference between the means of Length of Earthworm on Without Humic Acid and Humic Acid 1000 mg for Day 14.

TABLE:5.27

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	NOHD14	21.2	10	1.814	0.573
	H100D14	21.2	10	1.874	0.593
Pair 2	NOHD14	21.2	10	1.814	0.573
	H250D14	22.3	10	2.003	0.633
Pair 3	NOHD14	21.2	10	1.814	0.573
	H500D14	19	10	2.309	0.73
Pair 4	NOHD14	21.2	10	1.814	0.573
	H750D14	19.4	10	2.271	0.718
Pair 5	NOHD14	21.2	10	1.814	0.573
	H1000D14	19.9	10	1.524	0.482

TABLE:5.28**Paired Samples Correlations**

		N	Correlation	Sig.
Pair 1	NOHD14 & H100D14	10	0.935	0
Pair 2	NOHD14 & H250D14	10	0.961	0
Pair 3	NOHD14 & H500D14	10	0.955	0
Pair 4	NOHD14 & H750D14	10	0.95	0
Pair 5	NOHD14 & H1000D14	10	0.933	0

TABLE:5.29**Paired Samples Test**

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	NOHD14 - H100D14	0	0.667	0.211	-0.477	0.477	0	9	1
Pair 2	NOHD14 - H250D14	-1.1	0.568	0.18	-1.506	-0.694	-6.128	9	0
Pair 3	NOHD14 - H500D14	2.2	0.789	0.249	1.636	2.764	8.82	9	0
Pair 4	NOHD14 - H750D14	1.8	0.789	0.249	1.236	2.364	7.216	9	0
Pair 5	NOHD14 - H1000D14	1.3	0.675	0.213	0.817	1.783	6.091	9	0

Inference

Pair 1:

To test the hypothesis that Without Humic Acid (Mean = 21.20, Standard Deviation = 1.814) and Humic Acid means (Mean = 21.20, Standard Deviation = 1.874) were equal, paired sample t-test has been performed. The correlation value $r = .935$, $p = .009$, there exists a positive correlation between the variables. Since the sig value is 0.000, which is < 0.05 , reject null hypothesis. There is a significant difference between

the means of Length of Earthworm on Without Humic Acid and Humic Acid 100 mg for Day 7.

Pair 2:

To test the hypothesis that Without Humic Acid (Mean = 21.20, Standard Deviation = 1.814) and Humic Acid means (Mean = 22.30, Standard Deviation = 2.003) were equal, paired sample t-test has been performed. The correlation value $r = .961$, $p = .003$, there exists a perfect positive correlation between the variables. Since the sig value is 0.000, which is < 0.05 , reject null hypothesis. There is a significant difference between the means of Length of Earthworm on Without Humic Acid and Humic Acid 250 mg for Day 7.

Pair 3:

To test the hypothesis that No Humic Acid (Mean = 21.20, Standard Deviation = 1.814) and Humic Acid means (Mean = 19.00, Standard Deviation = 2.309) were equal, paired sample t-test has been performed. The correlation value $r = .955$, $p = .000$, there exists a perfect positive correlation between the variables. Since the sig value is 0.000, which is < 0.01 , reject null hypothesis. There is a significant difference between the means of Length of Earthworm on Without Humic Acid and Humic Acid 500 mg for Day 7.

Pair 4:

To test the hypothesis that Without Humic Acid (Mean = 21.20, Standard Deviation = 1.814) and Humic Acid means (Mean = 19.40, Standard Deviation = 2.271) were equal, paired sample t-test has been performed. The correlation value $r = .950$, $p = .005$, there exists a perfect positive correlation between the variables. Since the sig value is .000, which is < 0.05 , reject null hypothesis. There is a significant difference between the means of Length of Earthworm on Without Humic Acid and Humic Acid 750 mg for Day 7.

Pair 5:

To test the hypothesis that Without Humic Acid (Mean = 21.20, Standard Deviation = 1.814) and Humic Acid means (Mean = 19.90, Standard Deviation = 2.524) were equal, paired sample t-test has been performed. The correlation value $r = .933$, $p =$

.010, there exists a positive correlation between the variables. Since the sig value is 0.000, which is < 0.05 , reject null hypothesis. There is a significant difference between the means of Length of Earthworm on Without Humic Acid and Humic Acid 1000 mg for Day 7.

Paired Samples t-test for Day 21

Pair 1:

H₀1: There is no significant difference between the means of Length of Earthworm on Without Humic Acid and Humic Acid 100 mg for Day 21.

H₁1: There is significant difference between the means of Length of Earthworm on Without Humic Acid and Humic Acid 100 mg for Day 21.

Pair 2:

H₀1: There is no significant difference between the means of Length of Earthworm on Without Humic Acid and Humic Acid 250 mg for Day 21.

H₁1: There is significant difference between the means of Length of Earthworm on Without Humic Acid and Humic Acid 250 mg for Day 21.

Pair 3:

H₀1: There is no significant difference between the means of Length of Earthworm Without No Humic Acid and Humic Acid 500 mg for Day 21.

H₁1: There is significant difference between the means of Length of Earthworm on Without Humic Acid and Humic Acid 500 mg for Day 21.

Pair 4:

H₀1: There is no significant difference between the means of Length of Earthworm on Without Humic Acid and Humic Acid 750 mg for Day 21.

H₁1: There is significant difference between the means of Length of Earthworm on Without Humic Acid and Humic Acid 750 mg for Day 21.

Pair 5:

H₀1: There is no significant difference between the means of Length of Earthworm on Without Humic Acid and Humic Acid 1000 mg for Day 21.

H₁1: There is significant difference between the means of Length of Earthworm on Without Humic Acid and Humic Acid 1000 mg for Day 21.

TABLE: 5.30

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	NOHD21	21.8	10	1.814	0.573
	H100D21	22	10	1.563	0.494
Pair 2	NOHD21	21.8	10	1.814	0.573
	H250D21	22.8	10	1.874	0.593
Pair 3	NOHD21	21.8	10	1.814	0.573
	H500D21	19.5	10	1.841	0.582
Pair 4	NOHD21	21.8	10	1.814	0.573
	H750D21	20.3	10	2.312	0.731
Pair 5	NOHD21	21.8	10	1.814	0.573
	H1000D21	21	10	1.764	0.558

TABLE:5.31

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	NOHD21 & H100D21	10	0.94	0
Pair 2	NOHD21 & H250D21	10	0.935	0
Pair 3	NOHD21 & H500D21	10	0.965	0
Pair 4	NOHD21 & H750D21	10	0.97	0
Pair 5	NOHD21 & H1000D21	10	0.938	0

TABLE:5.32**Paired Samples Test**

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	NOHD21 - H100D21	-0.2	0.632	0.2	-0.652	0.252	-1	9	0.343
Pair 2	NOHD21 - H250D21	-1	0.667	0.211	-1.477	-0.523	-4.743	9	0.001
Pair 3	NOHD21 - H500D21	2.3	0.483	0.153	1.954	2.646	15.057	9	0
Pair 4	NOHD21 - H750D21	1.5	0.707	0.224	0.994	2.006	6.708	9	0
Pair 5	NOHD21 - H1000D21	0.8	0.632	0.2	0.348	1.252	4	9	0.003

Inference**Pair 1:**

To test the hypothesis that Without Humic Acid (Mean = 21.80, Standard Deviation = 1.814) and Humic Acid means (Mean = 22.00, Standard Deviation = 1.563) were equal, paired sample t-test has been performed. The correlation value $r = .940$, $p = .009$, there exists a positive correlation between the variables. Since the sig value is 0.000, which is < 0.05 , reject null hypothesis. There is a significant difference between the means of Length of Earthworm on Without Humic Acid and Humic Acid 100 mg for Day 7.

Pair 2:

To test the hypothesis that Without Humic Acid (Mean = 21.80, Standard Deviation = 1.814) and Humic Acid means (Mean = 22.80, Standard Deviation = 1.874) were equal, paired sample t-test has been performed. The correlation value $r = .935$, $p = .003$, there exists a perfect positive correlation between the variables. Since the sig value is 0.000, which is < 0.05 , reject null hypothesis. There is a significant difference between the means of Length of Earthworm on Without Humic Acid and Humic Acid 250 mg for Day 7.

Pair 3:

To test the hypothesis that No Humic Acid (Mean = 21.80, Standard Deviation = 1.814) and Humic Acid means (Mean = 19.50, Standard Deviation = 1.841) were equal, paired sample t-test has been performed. The correlation value $r = .965$, $p = .000$, there exists a perfect positive correlation between the variables. Since the sig value is 0.000, which is < 0.01 , reject null hypothesis. There is a significant difference between the means of Length of Earthworm on Without Humic Acid and Humic Acid 500 mg for Day 7.

Pair 4:

To test the hypothesis that Without Humic Acid (Mean = 21.80, Standard Deviation = 1.814) and Humic Acid means (Mean = 20.30, Standard Deviation = 2.312) were equal, paired sample t-test has been performed. The correlation value $r = .970$, $p = .005$, there exists a perfect positive correlation between the variables. Since the sig value is .000, which is < 0.05 , reject null hypothesis. There is a significant difference between the means of Length of Earthworm on Without Humic Acid and Humic Acid 750 mg for Day 7.

Pair 5:

To test the hypothesis that Without Humic Acid (Mean = 21.80, Standard Deviation = 1.814) and Humic Acid means (Mean = 21.00, Standard Deviation = 1.764) were equal, paired sample t-test has been performed. The correlation value $r = .938$, $p = .010$, there exists a positive correlation between the variables. Since the sig value is 0.000, which is < 0.05 , reject null hypothesis. There is a significant difference between the means of Length of Earthworm on Without Humic Acid and Humic Acid 1000 mg for Day 7.

5.6.2. WEIGHT STATISTICS:

Paired Samples t-test for Day 7

Pair 1:

H₀1: There is no significant difference between the means of Weight of Earthworm on Without Humic Acid and Humic Acid 100 mg for Day 7.

H₁1: There is significant difference between the means of Weight of Earthworm on Without Humic Acid and Humic Acid 100 mg for Day 7.

Pair 2:

H₀1: There is no significant difference between the means of Weight of Earthworm on Without Humic Acid and Humic Acid 250 mg for Day 7.

H₁1: There is significant difference between the means of Weight of Earthworm on Without Humic Acid and Humic Acid 250 mg for Day 7.

Pair 3:

H₀1: There is no significant difference between the means of Weight of Earthworm on Without Humic Acid and Humic Acid 500 mg for Day 7.

H₁1: There is significant difference between the means of Weight of Earthworm on Without Humic Acid and Humic Acid 500 mg for Day 7.

Pair 4:

H₀1: There is no significant difference between the means of Weight of Earthworm on Without Humic Acid and Humic Acid 750 mg for Day 7.

H₁1: There is significant difference between the means of Weight of Earthworm on Without Humic Acid and Humic Acid 750 mg for Day 7.

Pair 5:

H₀1: There is no significant difference between the means of Weight of Earthworm on Without Humic Acid and Humic Acid 1000 mg for Day 7.

H₁1: There is significant difference between the means of Weight of Earthworm on Without Humic Acid and Humic Acid 1000 mg for Day 7.

TABLE:5.33**Paired Samples Statistics**

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	NOHUMD7	1.002	10	0.13935	0.04407
	HUM100D7	0.955	10	0.0912	0.02884
Pair 2	NOHUMD7	1.002	10	0.13935	0.04407
	HUM250D7	1.027	10	0.12239	0.0387
Pair 3	NOHUMD7	1.002	10	0.13935	0.04407
	HUM500D7	0.879	10	0.10651	0.03368
Pair 4	NOHUMD7	1.002	10	0.13935	0.04407
	HUM750D7	0.927	10	0.12401	0.03922
Pair 5	NOHUMD7	1.002	10	0.13935	0.04407
	HUM1000D7	0.956	10	0.10255	0.03243

TABLE:5.34**Paired Samples Correlations**

		N	Correlation	Sig.
Pair 1	NOHUMD7 & HUM100D7	10	0.775	0.009
Pair 2	NOHUMD7 & HUM250D7	10	0.828	0.003
Pair 3	NOHUMD7 & HUM500D7	10	0.935	0
Pair 4	NOHUMD7 & HUM750D7	10	0.8	0.005
Pair 5	NOHUMD7 & HUM1000D7	10	0.767	0.01

TABLE:5.35**Paired Samples Test**

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	NOHUMD7 - HUM100D7	0.047	0.0897	0.02836	-0.01717	0.11117	1.657	9	0.132
Pair 2	NOHUMD7 - HUM250D7	-0.025	0.07835	0.02478	-0.08105	0.03105	-1.009	9	0.339
Pair 3	NOHUMD7 - HUM500D7	0.123	0.05478	0.01732	0.08381	0.16219	7.1	9	0
Pair 4	NOHUMD7 - HUM750D7	0.075	0.08462	0.02676	0.01446	0.13554	2.803	9	0.021
Pair 5	NOHUMD7 - HUM1000D7	0.046	0.08947	0.02829	-0.018	0.11	1.626	9	0.138

Inference**Pair 1:**

To test the hypothesis that Without Humic Acid (Mean = 1.0020, Standard Deviation = .13935) and Humic Acid means (Mean = .9550, Standard Deviation = .09120) were equal, paired sample t-test has been performed. The correlation value $r = .775$, $p = .009$, there exists a positive correlation between the variables. Since the sig value is .132, which is > 0.05 , accept null hypothesis. There is no significant difference between the means of Weight of Earthworm on Without Humic Acid and Humic Acid 100 mg for Day 7.

Pair 2:

To test the hypothesis that Without Humic Acid (Mean = 1.0020, Standard Deviation = .13935) and Humic Acid means (Mean = 1.0270, Standard Deviation = .12239) were

equal, paired sample t-test has been performed. The correlation value $r = .828$, $p = .003$, there exists a perfect positive correlation between the variables. Since the sig value is .339, which is > 0.05 , accept null hypothesis. There is no significant difference between the means of Weight of Earthworm on Without Humic Acid and Humic Acid 250 mg for Day 7.

Pair 3:

To test the hypothesis that No Humic Acid (Mean = 1.0020, Standard Deviation = .13935) and Humic Acid means (Mean = .8790, Standard Deviation = .10651) were equal, paired sample t-test has been performed. The correlation value $r = .935$, $p = .000$, there exists a perfect positive correlation between the variables. Since the sig value is 0.000, which is < 0.01 , reject null hypothesis. There is a significant difference between the means of Weight of Earthworm on Without Humic Acid and Humic Acid 500 mg for Day 7.

Pair 4:

To test the hypothesis that Without Humic Acid (Mean = 1.0020, Standard Deviation = .13935) and Humic Acid means (Mean = .9270, Standard Deviation = .12401) were equal, paired sample t-test has been performed. The correlation value $r = .800$, $p = .005$, there exists a perfect positive correlation between the variables. Since the sig value is .021, which is < 0.05 , reject null hypothesis. There is a significant difference between the means of Weight of Earthworm on Without Humic Acid and Humic Acid 750 mg for Day 7.

Pair 5:

To test the hypothesis that Without Humic Acid (Mean = 1.0020, Standard Deviation = .13935) and Humic Acid means (Mean = .9560, Standard Deviation = .10255) were equal, paired sample t-test has been performed. The correlation value $r = .767$, $p = .010$, there exists a positive correlation between the variables. Since the sig value is 0.138, which is > 0.05 , accept null hypothesis. There is no significant difference between the means of Weight of Earthworm on Without Humic Acid and Humic Acid 1000 mg for Day 7.

Paired Samples t-test for Day 14

Pair 1:

H₀1: There is no significant difference between the means of Weight of Earthworm on Without Humic Acid and Humic Acid 100 mg for Day 14.

H₁1: There is significant difference between the means of Weight of Earthworm on Without Humic Acid and Humic Acid 100 mg for Day 14.

Pair 2:

H₀1: There is no significant difference between the means of Weight of Earthworm on Without Humic Acid and Humic Acid 250 mg for Day 14.

H₁1: There is significant difference between the means of Weight of Earthworm on Without Humic Acid and Humic Acid 250 mg for Day 14.

Pair 3:

H₀1: There is no significant difference between the means of Weight of Earthworm on Without Humic Acid and Humic Acid 500 mg for Day 14.

H₁1: There is significant difference between the means of Weight of Earthworm on Without Humic Acid and Humic Acid 500 mg for Day 14.

Pair 4:

H₀1: There is no significant difference between the means of Weight of Earthworm on Without Humic Acid and Humic Acid 750 mg for Day 14.

H₁1: There is significant difference between the means of Weight of Earthworm on Without Humic Acid and Humic Acid 750 mg for Day 14.

Pair 5:

H₀1: There is no significant difference between the means of Weight of Earthworm on Without Humic Acid and Humic Acid 1000 mg for Day 14.

H₁1: There is significant difference between the means of Weight of Earthworm on Without Humic Acid and Humic Acid 1000 mg for Day 14.

TABLE: 5.36**Paired Samples Statistics**

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	NOHUMD14	1.23	10	0.15727	0.04973
	HUM100D14	1.13	10	0.12347	0.03904
Pair 2	NOHUMD14	1.23	10	0.15727	0.04973
	HUM250D14	1.252	10	0.19378	0.06128
Pair 3	NOHUMD14	1.23	10	0.15727	0.04973
	HUM500D14	0.968	10	0.13579	0.04294
Pair 4	NOHUMD14	1.23	10	0.15727	0.04973
	HUM750D14	1.004	10	0.18289	0.05784
Pair 5	NOHUMD14	1.23	10	0.15727	0.04973
	HUM1000D14	1.096	10	0.12331	0.03899

TABLE: 5.37**Paired Samples Correlations**

		N	Correlation	Sig.
Pair 1	NOHUMD14 & HUM100D14	10	0.806	0.005
Pair 2	NOHUMD14 & HUM250D14	10	0.759	0.011
Pair 3	NOHUMD14 & HUM500D14	10	0.825	0.003
Pair 4	NOHUMD14 & HUM750D14	10	0.713	0.021
Pair 5	NOHUMD14 & HUM1000D14	10	0.707	0.022

TABLE: 5.38**Paired Samples Test**

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	NOHUMD14 - HUM100D14	0.1	0.09321	0.02948	0.03332	0.16668	3.392	9	0.008
Pair 2	NOHUMD14 - HUM250D14	-0.022	0.12656	0.04002	-0.11254	0.06854	-0.55	9	0.596
Pair 3	NOHUMD14 - HUM500D14	0.262	0.08904	0.02816	0.1983	0.3257	9.305	9	0
Pair 4	NOHUMD14 - HUM750D14	0.226	0.131	0.04142	0.13229	0.31971	5.456	9	0
Pair 5	NOHUMD14 - HUM1000D14	0.134	0.11187	0.03538	0.05397	0.21403	3.788	9	0.004

Inference**Pair 1:**

To test the hypothesis that Without Humic Acid (Mean = 1.2300, Standard Deviation = .15727) and Humic Acid means (Mean = 1.1300, Standard Deviation = .12347) were equal, paired sample t-test has been performed. The correlation value $r = .806$, $p = .005$, there exists a perfect positive correlation between the variables. Since the sig value is .008, which is < 0.01 , reject null hypothesis. There is a significant difference between the means of Weight of Earthworm on Without Humic Acid and Humic Acid 100 mg for Day 14.

Pair 2:

To test the hypothesis that Without Humic Acid (Mean = 1.2300, Standard Deviation = .15727) and Humic Acid means (Mean = 1.2520, Standard Deviation = .19378) were equal, paired sample t-test has been performed. The correlation value $r = .759$, $p = .011$, there exists a positive correlation between the variables. Since the sig value is

.596, which is > 0.05 , accept null hypothesis. There is no significant difference between the means of Weight of Earthworm on Without Humic Acid and Humic Acid 250 mg for Day 14.

Pair 3:

To test the hypothesis that No Humic Acid (Mean = 1.2300, Standard Deviation = .15727) and Humic Acid means (Mean = .9680, Standard Deviation = .13579) were equal, paired sample t-test has been performed. The correlation value $r = .825$, $p = .003$, there exists a perfect positive correlation between the variables. Since the sig value is 0.000, which is < 0.01 , reject null hypothesis. There is a significant difference between the means of Weight of Earthworm on Without Humic Acid and Humic Acid 500 mg for Day 14.

Pair 4:

To test the hypothesis that Without Humic Acid (Mean = 1.2300, Standard Deviation = .15727) and Humic Acid means (Mean = 1.0040, Standard Deviation = .18289) were equal, paired sample t-test has been performed. The correlation value $r = .713$, $p < .021$, there exists a positive correlation between the variables. Since the sig value is 0.000, which is < 0.01 , reject null hypothesis. There is a significant difference between the means of Weight of Earthworm on Without Humic Acid and Humic Acid 750 mg for Day 14.

Pair 5:

To test the hypothesis that Without Humic Acid (Mean = 1.2300, Standard Deviation = .15727) and Humic Acid means (Mean = 1.0960, Standard Deviation = .12331) were equal, paired sample t-test has been performed. The correlation value $r = .707$, $p < .022$, there exists a positive correlation between the variables. Since the sig value is 0.004, which is < 0.01 , reject null hypothesis. There is a significant difference between the means of Weight of Earthworm on Without Humic Acid and Humic Acid 1000 mg for Day 14.

Paired Samples t-test for Day 21

Pair 1:

H₀1: There is no significant difference between the means of Weight of Earthworm on Without Humic Acid and Humic Acid 100 mg for Day 21.

H₁1: There is significant difference between the means of Weight of Earthworm on Without Humic Acid and Humic Acid 100 mg for Day 21.

Pair 2:

H₀1: There is no significant difference between the means of Weight of Earthworm on Without Humic Acid and Humic Acid 250 mg for Day 21.

H₁1: There is significant difference between the means of Weight of Earthworm on Without Humic Acid and Humic Acid 250 mg for Day 21.

Pair 3:

H₀1: There is no significant difference between the means of Weight of Earthworm Without No Humic Acid and Humic Acid 500 mg for Day 21.

H₁1: There is significant difference between the means of Weight of Earthworm on Without Humic Acid and Humic Acid 500 mg for Day 21.

Pair 4:

H₀1: There is no significant difference between the means of Weight of Earthworm on Without Humic Acid and Humic Acid 750 mg for Day 21.

H₁1: There is significant difference between the means of Weight of Earthworm on Without Humic Acid and Humic Acid 750 mg for Day 21.

Pair 5:

H₀1: There is no significant difference between the means of Weight of Earthworm on Without Humic Acid and Humic Acid 1000 mg for Day 21.

H₁1: There is significant difference between the means of Weight of Earthworm on Without Humic Acid and Humic Acid 1000 mg for Day 21.

TABLE: 5.39**Paired Samples Statistics**

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	NOHUMD21	1.307	10	0.21302	0.06736
	HUM100D21	1.337	10	0.1529	0.04835
Pair 2	NOHUMD21	1.307	10	0.21302	0.06736
	HUM250D21	1.452	10	0.14756	0.04666
Pair 3	NOHUMD21	1.307	10	0.21302	0.06736
	HUM500D21	1.126	10	0.20151	0.06372
Pair 4	NOHUMD21	1.307	10	0.21302	0.06736
	HUM750D21	1.211	10	0.21825	0.06902
Pair 5	NOHUMD21	1.307	10	0.21302	0.06736
	HUM1000D21	1.317	10	0.14553	0.04602

TABLE: 5.40**Paired Samples Correlations**

		N	Correlation	Sig.
Pair 1	NOHUMD21 & HUM100D21	10	0.543	0.105
Pair 2	NOHUMD21 & HUM250D21	10	0.696	0.026
Pair 3	NOHUMD21 & HUM500D21	10	0.771	0.009
Pair 4	NOHUMD21 & HUM750D21	10	0.734	0.016
Pair 5	NOHUMD21 & HUM1000D21	10	0.366	0.298

TABLE: 5.41**Paired Samples Test**

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	NOHUMD21 - HUM100D21	-0.03	0.1827	0.05777	-0.16069	0.10069	-0.519	9	0.616
Pair 2	NOHUMD21 - HUM250D21	-0.145	0.15306	0.0484	-0.25449	-0.03551	-2.996	9	0.015
Pair 3	NOHUMD21 - HUM500D21	0.181	0.14083	0.04453	0.08026	0.28174	4.064	9	0.003
Pair 4	NOHUMD21 - HUM750D21	0.096	0.15749	0.0498	-0.01666	0.20866	1.928	9	0.086
Pair 5	NOHUMD21 - HUM1000D21	-0.01	0.20939	0.06622	-0.15979	0.13979	-0.151	9	0.883

Inference**Pair 1:**

To test the hypothesis that Without Humic Acid (Mean = 1.3070, Standard Deviation = .21302) and Humic Acid means (Mean = 1.3370, Standard Deviation = .15290) were equal, paired sample t-test has been performed. The correlation value $r = .543$, $p = .105$, there exists a positive correlation between the variables. Since the sig value is 0.616, which is > 0.05 , accept null hypothesis. There is no significant difference between the means of Weight of Earthworm on Without Humic Acid and Humic Acid 100 mg for Day 21.

Pair 2:

To test the hypothesis that Without Humic Acid (Mean = 1.3070, Standard Deviation = .21302) and Humic Acid means (Mean = 1.4520, Standard Deviation = .14756) were equal, paired sample t-test has been performed. The correlation value $r = .696$, $p = .026$, there exists a positive correlation between the variables. Since the sig value is

0.015, which is < 0.05 , reject null hypothesis. There is a significant difference between the means of Weight of Earthworm on Without Humic Acid and Humic Acid 250 mg for Day 21.

Pair 3:

To test the hypothesis that No Humic Acid (Mean = 1.3070, Standard Deviation = .21302) and Humic Acid means (Mean = 1.1260, Standard Deviation = .20151) were equal, paired sample t-test has been performed. The correlation value $r = .771$, $p = .009$, there exists a positive correlation between the variables. Since the sig value is 0.003, which is < 0.01 , reject null hypothesis. There is a significant difference between the means of Weight of Earthworm on Without Humic Acid and Humic Acid 500 mg for Day 21.

Pair 4:

To test the hypothesis that Without Humic Acid (Mean = 1.3070, Standard Deviation = .21302) and Humic Acid means (Mean = 1.2110, Standard Deviation = .21825) were equal, paired sample t-test has been performed. The correlation value $r = .734$, $p = .016$, there exists a positive correlation between the variables. Since the sig value is .086, which is > 0.05 , accept null hypothesis. There is no significant difference between the means of Weight of Earthworm on Without Humic Acid and Humic Acid 750 mg for Day 21.

Pair 5:

To test the hypothesis that Without Humic Acid (Mean = 1.3070, Standard Deviation = .21302) and Humic Acid means (Mean = 1.3170, Standard Deviation = .14553) were equal, paired sample t-test has been performed. The correlation value $r = .366$, $p = .298$, there exists a positive correlation between the variables. Since the sig value is .883, which is > 0.05 , accept null hypothesis. There is no significant difference between the means of Weight of Earthworm on Without Humic Acid and Humic Acid 1000 mg for Day 21.

5.7. BEHAVIOURAL STUDIES:

It was observed that all the earthworms subjected to humic acid were active and had fast motion, proving that the humic acid did not affect their metabolism and did not cause any changes in their behaviour.

5.8. BIOACCUMULATION:

TABLE: 5.42: ICP-OES Results for Earthworms

Earthworm at concentration	Element symbol and Wavelength (nm)	Weight of sample in gms / Volume in ml	Dilution Factor	
Control	Cr 267.716	0.3081g/50ml	1	0.052 mg/L
	Pb 220.353	„	1	BDL
Conc-1	Cr 267.716	0.3095g/50ml	1	0.026 mg/L
	Pb 220.353	„	1	BDL
Conc-2	Cr 267.716	0.3775g/50ml	1	0.007 mg/L
	Pb 220.353	„	1	BDL
Conc-3	Cr 267.716	0.4001g/50ml	1	0.029 mg/L
	Pb 220.353	„	1	0.036 mg/L
Conc-4	Cr 267.716	0.3518g/50ml	1	0.004 mg/L
	Pb 220.353	„	1	0.035 mg/L
Conc-5	Cr 267.716	0.3740g/50ml	1	0.022 mg/L
	Pb 220.353	„	1	BDL

Inductively coupled plasma-optical emission spectroscopy (ICP-OES) was done for the earthworms that had been subjected to different concentrations of humic acid. The analysis was done at SAIF, IIT-M for the elements lead and chromium.

It can be observed that minimal amounts of chromium were found in all the earthworms, including the earthworms that were not subjected to humic acid. On the other hand, lead is below the detection limit in most of cases. Only the earthworms that were subjected to 0.05% (Conc-3) and 0.075% (Conc-4) had trace amounts of

lead. The amount of lead and chromium found in the control was 0.052 mg/L of chromium and lead was below the required detection level. The amount of lead and chromium found in concentration-1 was 0.026 mg/L of chromium and lead was below the required detection level. The amount of lead and chromium found in concentration-2 was 0.007 mg/L of chromium and lead was below the required detection level. The amount of lead and chromium found in concentration-3 were 0.029 mg/L of chromium and 0.036 mg/L of lead. The amount of lead and chromium found in concentration-4 were 0.004 mg/L of chromium and 0.035 mg/L of lead. The amount of lead and chromium found in concentration-5 were 0.029 mg/L of chromium and 0.036 mg/L of lead.

CHAPTER 6: SUMMARY AND CONCLUSION

6.1.SUMMARY

Humic acid was prepared in our lab facility from lignite with a series of steps involving weighing, the addition of KOH, sonication, centrifugation, and drying. The obtained humic acid was then used for our ecotoxicity studies, where the humic acid was added to 15 trays of earthworms at 5 different concentrations. The humic acid was given out for ATR-IR and SEM analysis at the SAIF, IIT Madras facility. In addition, the lignite sample was also given for an ATR-IR analysis at the same facility. The setup for our project was done in an open environment in 18 trays that were 13 inches wide and 3 inches deep and an adequate amount of soil was added to each tray. We had 3 trays for control, 3 for concentration-1 and likewise up to concentration-5. We had a total of 180 earthworms, 10 earthworms were allowed to inhabit a tray. These trays were then covered by a mesh to protect them from predators. 5 different concentrations of humic acid were prepared 35mg, 875mg, 1750mg, 2625mg and 3500mg. Then ecotoxicity studies such as morphology, survival, fecundity, behavioural, and bioaccumulation were carried out. The length and the mass for morphology were recorded on the 1st, 7th, 14th, and 21st days. Survivability and behaviour were also noticed during the 21 days. By the end of the 21st-day juveniles were observed in trays of all concentrations. Soil from control and concentration-5 was given at the National Agro Foundation for basic soil and organic fertilizer testing. Earthworms were given out for ICP-OES at SAIF, IIT Madras for analysis.

6.2 CONCLUSION

It can be noticed that all 18 trays had a uniform growth with very minor differences. All 180 earthworms survived the 21 days studies and it was observed that reproduction had taken place. Behavioural changes or any sort of abnormalities were not observed. Proving that the different concentrations of humic acid did not affect their growth in any way. We can thus conclude that humic acid is not toxic.

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