ISOLATION AND CHARACTERIZATION OF SOIL BACTERIA CAPABLE OF DEGRADING METRIBUZIN IN SUGARCANE FARMS OF WESTERN KENYA

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A Thesis Submitted to Graduate School in Partial Fulfilment for the Requirements of Master of Science Degree in Environmental Science of Egerton University

EGERTON UNIVERSITY

November, 2016
DECLARATION AND RECOMMENDATION

DECLARATION

I hereby declare that this is my original work and has not been presented in this or any university for the award of a degree

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RECOMMENDATION

This thesis has been submitted for examination with our approval as supervisors.

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DEDICATION

This thesis is dedicated to my husband Mac, my daughter Ray and son Ian.
ACKNOWLEDGEMENT
I would like to thank God for giving me the strength and health to pursue this study. I would like to express my sincere gratitude to my supervisors Dr. A. W. Muia, Dr. Wilkister Moturi and Dr. Anastasia Ngigi for professional guidance. I highly regard Mr Francis Oringe, the Agronomist of Nzoia sugar Company for giving me assistance in soil sampling. Many thanks go to Mr Stanely Babikha, the Nucleus Estate Manager of Nzoia Sugar Company, for facilitating sampling in the estates and Mr Aggrey Khaemba, the supervisor in-charge of spraying for giving us information on herbicides history and helping in identification of farms during sampling. Regards to Mr. Caleb Luvonga and entire Biochemistry department of Kenya Bureau of Standards for the technical assistance in HPLC analysis. Mr Cyrus Kimani of KALRO Njoro together with Dr. Miriam Charimbu of Egerton University (CHS Dept) for assistance in molecular analysis of isolates. I am also thankful for Egerton University for giving me the opportunity to pursue my study. I thank my family for love, support and the encouragement they provided to me during the time of my study.
ABSTRACT

Nzoia River Drainage Basin is a major sugar production region in Kenya. Various pesticides are applied in this area to control weeds and boost sugar productivity. However, use of herbicides for weed control leads to increased chemical loads in the environment whose effects could be disastrous to the biotic component. The use of pesticide adapted microorganisms in the degradation and detoxification of many toxic xenobiotics, especially pesticides, is an efficient tool for the decontamination of polluted environments. The main objective of this work was to isolate and identify soil bacteria capable of degrading metribuzin, a commonly used weed killer in sugarcane farms. Five farms with history of metribuzin application were purposively identified for soil sampling. Random soil sampling was used to obtain samples from a depth of 0 – 10cm in November 2013. A composite sample was used in isolation of the bacteria. Influence of temperature, pH, nitrogen and phosphorous on growth of the isolated bacteria was also tested. Experimental design was used to carry out the experiments in the laboratory. Mineral salts media containing metribuzin as the sole carbon source was used to culture and selectively isolate metribuzin degrading bacteria. Growth of the bacteria in the medium measured as absorbance at OD$_{600nm}$ for various time intervals was an indication of tolerance to the herbicide and ability to utilize metribuzin as a carbon source. HPLC method was used to determine ability of the bacteria to degrade metribuzin and assess the metabolites after the 21 day incubation period. Molecular analysis was carried out by DNA extraction from each isolate and subjected to PCR using 16S primers. Sequences and blast results were compared to relevant data bases. ANOVA and separation of means using LSD at $p \leq 0.05$ was used to analyse data. Seven different bacteria isolates with metribuzin degrading potential were coded NZ453A, NZ454B, NZ453C, NZ543A, NZ543B, NZ8070 and NZ1110. They were subjected to morphological, cultural, biochemical and molecular characterization. Results also revealed that temperature, pH, nitrogen and phosphorous had different influence on the specific bacteria but generally, $35^\circ C$, pH 9, nitrogen and phosphorous concentrations of 7.5g/L recorded highest growth on most of the isolates. The isolates degraded more than 93% of metribuzin. The seven isolated bacteria were identified as Planococcus sp., Burkholderia cepacia, Pseudomonas sp., Bacillus sp., Arthrobacter sp., and Staphylococcus sp., all of which have been previously associated with degradation of recalcitrant compounds in the environment. This indicates that Nzoia sugarcane farms consist of different metribuzin degrading bacteria, which can grow in
different physical chemical conditions. They can be multiplied and further developed for bioremediation or bioaugmentation of metribuzin contaminated sites.

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<th>Abbreviation</th>
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<tbody>
<tr>
<td>CCME</td>
<td>Canadian Council of Ministers of the Environment.</td>
</tr>
<tr>
<td>DA</td>
<td>Desamino</td>
</tr>
<tr>
<td>DADK</td>
<td>Desamino-diketo</td>
</tr>
<tr>
<td>DK</td>
<td>Diketo</td>
</tr>
<tr>
<td>EXTOXNET</td>
<td>Extension Toxicological Network.</td>
</tr>
<tr>
<td>HPLC</td>
<td>High Performance Liquid Chromatography.</td>
</tr>
<tr>
<td>HSDB</td>
<td>Hazardous Substances DataBank.</td>
</tr>
<tr>
<td>KESREF</td>
<td>Kenya Sugar Research Foundation.</td>
</tr>
<tr>
<td>NRDB</td>
<td>Nzoia River Drainage Basin.</td>
</tr>
<tr>
<td>NSF</td>
<td>Nzoia Sugar Farm</td>
</tr>
<tr>
<td>OMAF</td>
<td>Ontario Ministry of Agriculture and Food</td>
</tr>
<tr>
<td>OMOE</td>
<td>Ontario Ministry of Environment.</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction.</td>
</tr>
<tr>
<td>RTECS</td>
<td>Registry of Toxic Effects of Chemical Substances</td>
</tr>
<tr>
<td>USEPA</td>
<td>United States Environmental Protection Agency</td>
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