PRELIMINARY STUDIES ON THE BIOSORPTIVE VARIATIONS IN MONOCOTYLEDONOUS AND DICOTYLEDONOUS PLANTS

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Abstract - The role of plants in the biosorption of pollutants in both soil and aquatic environment is important towards developing unique bioremediation strategies. However, till date selection of these plants has mostly relied on previous studies and their choice of plants which renders phytoremediation strategies to be most loosely based on anecdotal evidence rather than a holistic strategy that considers the role of the different vascularized bundle systems between the two main plant classifications: monocotyledonous and dicotyledonous plant. This study is a preliminary study that attempts to study plants biosorptive efficiency on the basis of their structures, using organic and inorganic pollutants in soil with the goal of determining if the differences in their structure play a role in their efficiency.

Keywords - Bioremediation, Phytoremediation, Biosorption, Vascular bundles, Soil, Pollution.

I. INTRODUCTION

Pollution is regarded as one of the greatest challenges in modern times, probably its most visible effects is the altering of almost all geochemical cycles and concomitantly the biochemical balance of elements in both aquatic and terrestrial environments [1]. There are certain metals that act as micronutrients for the growth of plants, animals and human beings when present in trace amounts, namely, potassium, copper, zinc, manganese and cobalt, whereas, others such as cadmium, arsenic, mercury and chromium can have detrimental effects even in trace quantities [2].Several industries including agriculture, mining, petrochemical and allied manufacturing all contribute to environmental pollution [3,4]. With Petroleum contamination, some of the constituting compounds such as saturated and unsaturated straight-chain compounds are readily degraded and integrated into the photosynthetic pathway, however, the aromatic hydrocarbons such as benzene, anthracene, phenanthrene and benzo[a]pyrene are considered xenobiotic and are recalcitrant to degradation [5]. Petroleum pollution usually occurs due to natural oil seepages and accidental spillages [6, 7, 8, 9].

The entry of pollutants in soil and water has the concomitant effect of a negative integration into the food chain with the associated detrimental effects on all organisms as a result of biosorption and bioaccumulation in living systems which leads to diseases from toxemia and syndromes in time from mutagenic effects that can be passed to off springs [10]. Furthermore, the mobility of these pollutants leads to their seepage into ground water reserves reducing the quality of this important source of potable water. Mohammed and Normala (2015) [11], revealed that the factors that influence the accumulation of metal ions in the food web include the concentration of heavy metals in the soil, soil properties, sources of heavy metals, the degree and extent of uptake by plants and the extent of absorption

In recent times, the biological approach to remediation has gained pre-eminence due to environmental and cost considerations. Both the physical and chemical approaches to remediation tend to have associated drawbacks that has necessitated the utilization of bioremediation [12]. Bioremediation employs microorganisms such as bacteria [13, 14, 15, 16], fungi [17,18, 19, 20] algae [21, 22, 23] and plants in the clean-up of pollutants from the environment [24, 25, 26]. However, it comes with its own drawbacks which include longer process times [27], sometimes the transformation process may render the initial contaminants, more unstable and toxic than their parent compounds [28, 29] and often the introduction of non-indigenous microorganisms or plant species may further disrupt the ecological balance and biodiversity of the environment being bio-remediated [30] as the addition of nutrients (biostimulation) may promote the increased growth of these organisms being exploited for remediation purposes. Nevertheless, bioremediation has gained popularity as an effective strategy for environmental clean-up of pollution.

One form of bioremediation is the application of plants for the removal of pollutants from soil and/or the transformation that may lead to the degradation of the toxic pollutants to less harmful compounds. This process is referred to as phytoremediation [31]. However, the ability to accumulate compounds varies significantly amongst species and cultivars as a consequence of the difference in the mechanisms of ion uptake, often determined on the basis of morphology, anatomy, physiology and genetic composition of the various plants [32]. Moreover, the microbial-root symbiotic interactions specific to certain plant species are also contributing factors to element uptake and assimilatory efficiency in these plants [33]. Several studies have demonstrated plant phytoremediation of organic and inorganic pollutants

[34, 35, 36, 37, 38, 39]. However, information is sparse on determining element uptake as it relates to the structural differences in the vascular bundle arrangements in different plants. Therefore, this study will provide preliminary findings by observing trends in biosorption and its effects on growth, towards the elucidation of uptake of organic and inorganic compounds as it relates to the primary structure of plants.

II. DETAILS EXPERIMENTAL

2.1. Materials and Procedures

Sample (Plants) Selection

Four types of plant seeds were procured from Indigo Nursery, Midrand, South Africa. These included Bermuda grass (Cnyodondactylon) and Kikuyu grass (Pennisetumclandestinum) which represented the monocotyledonous plants and sunflower (Helianthus annuus) and hollyhock (Alcearosa) this represented the dicotyledonous plants.

Soil Sample Preparation Selection

Potting soil of garden variety was used for experiments and was also procured from Indigo Nursery, Midrand, South Africa. All soil used for main experiment was autoclaved with 10% (w/w) of water for moisture. Controls were set up using non-autoclaved soil.

To prepare soil sample simulating organic loading: Garage bought petrol from the Midrand area was sterilised using syringe pump facilitated 0.45um filters. This was used to contaminate soil to an approximate 4000 ppm concentration. The soil bed bottom was protected by plastic bag and a layer of sterile vermiculite to reduce run off, soil was hand-mixed with petrol to homogenise before it was poured on this bedding. This soil was left to volatilise for 2 weeks in the greenhouse, before seeds were introduced into the bed of soil [40].

To prepare soil sample simulating inorganic loading: Cadmium nitrate was diluted and mixed with soil before sterilisation to achieve an approximate 3 ppm concentration. The soil bed bottom was protected by plastic bag and a layer of sterile vermiculite to reduce run off. This soil was left to rest for 2 weeks in the greenhouse, before seeds were introduced into the bed of soil.

Seed Cultivation and Maintenance

The plant boxes were partitioned into nine squares for each plant type with an approximate 12 cm spacing between each square; each space was planted with 3 seeds of same plant species with an approximately 4 cm spacing and to a depth of 3 cm. This was to ensure the possibility of 27 replicates of each plant type for each of the different treatments (organic and inorganic contaminated but sterile and non-contaminated non-sterile soils). It was necessary to soak Alcearosea for 24 hours to halt dormancy and induce germination [41]. All other plant species used did not require this treatment and were planted directly. After cultivating the watering strategy for the duration of the experiment was the addition 10% (w/w) of double distilled water to the soil once every two days.

Sampling and Analysis of Plants

The quadrant sampling technique as described by Preen (1995) [42] was adapted for sampling each plant type for each treatment and its controls. It should be noted that this would have guaranteed a sampling size of 8 for each treatment. However, because the lowest quantity of plants that germinated was three all treatments were sampled in triplicates. Metric measurements using a vernier caliper was done weekly for stems and leaves, whilst observations were recorded for colour changes. Similarly, at the end of the experiment period, plants were up-rooted and their roots (length) were washed with distilled water and measured. Total mass of 3 individual plants were also measured for each treatment. Several cross-sections were cut from both the roots and stems of randomly selected plant replicates and were stained using both 0.1% methylene blue for approximately three minutes and safranin for 1 minute [43]. These stained sections were dried and mounted on glass slides with a drop of glycerine and a cover slip was placed before it was viewed using an Olympus CX41TM Light Microscope at X4 and X10 objective magnifications

III. RESULTS AND DISCUSSION

3.1. Results

Seed Cultivation and Maintenance

A total of 27 replicates was planted for each plant type in each treatment. In the petroleum (organic) contaminated soil, none of the seeds for Bermuda grass germinated. However, both the cadmium (inorganic) contaminated soil and controls set-up showed a 15% germination of seeds. Kikuyu grass demonstrated great number of seed germination with cadmium contaminated soil (96% germination) as compared to the 22% germination observed in petroleum-contaminated soil.

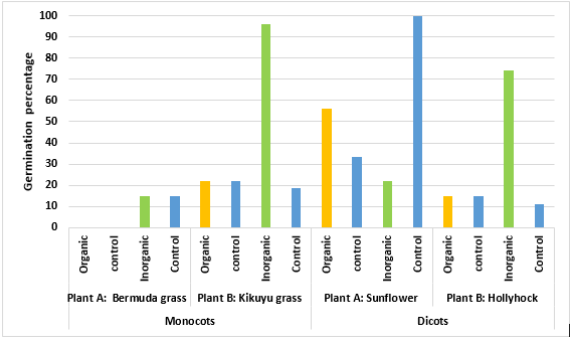


Figure 1: Comparison of plant germination in monocots and dicots in organic and inorganic contaminated soils

With the dicot plant species used all seeds had varying percentages of seeds germination. The control set up for Sunflower showed 100% germination. In petroleum-contaminated soil, approximately 56% of the Sunflower seeds germinated; however only 22% of the seeds planted in cadmium contaminated soil germinated. In comparison, 74% of Hollyhock germinated in cadmium contaminated, as compared with approximately 15% germination observed in petroleum-contaminated soil.

Sampling and Analysis of Plants

Bermuda grass showed no observable growth in the 6 weeks' duration of the experiment. Kikuyu grass demonstrated comparatively better growth (< 80mm shoot length) and tolerance of cadmium pollution as compared to its growth in petroleum-contaminated soil. However, both sunflower and hollyhock showed significant growth under both conditions, with sunflower showing the best tolerance in petroleum-contaminated soil and reaching a height of >110 mm during the 6 weeks.

Furthermore, chlorosis was observed in a significant number of plants growing in both types of contaminated soils.

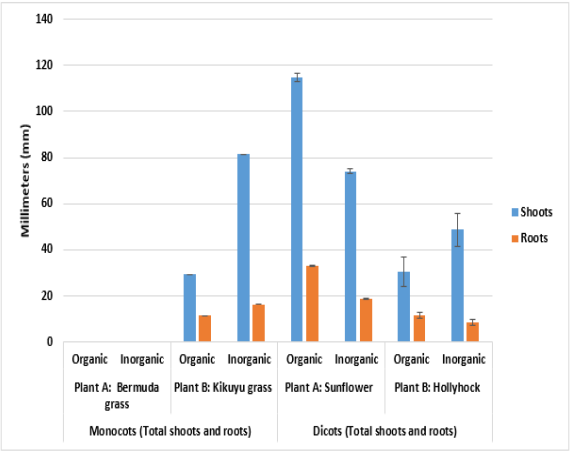


Figure 2: Comparison of Plants total shoot and root growth in the 6 weeks' duration of exposure to organic and inorganic contaminated soils

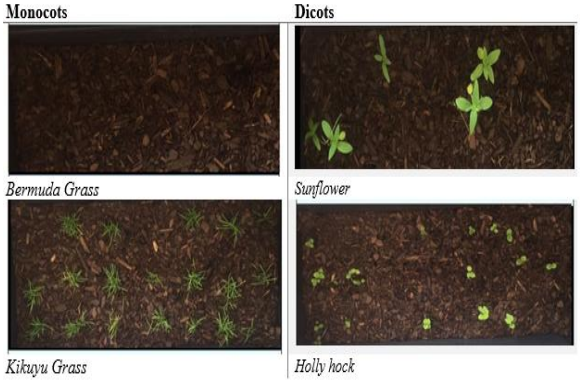


Table 1: Monocots and Dicots growth on plant bed after 3 weeks

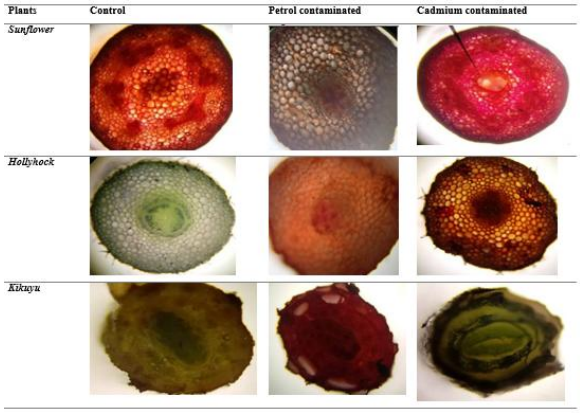


Table 2: Cross-sections of plants exposed to petrol and cadmium growth after 6 weeks viewed at X100 magnification

These pictures showed vascular bundles of plants after phytoremediation, note the darkened centres (xylem and phloem) of nearly all cross sections of the stem.

3.2. Discussion

An observational study was done with four different plant species to investigate possible correlation between structural and morphology differences in their biosorption abilities of pollutants in soil. The selection of these plant species were based on literature searches [44, 45, 46, 47, 48, 49, 50, 51, 52] that showed their previous applications in phytoremediation studies. The seeds of these four plant species were introduced to contaminated soil (petrol and cadmium contamination). It was observed that in the petrol contaminated soil none of the seeds for Bermuda grass germinated, however all other plant species seeds germinated with varying proportions. Similar observations were made by Mackinnon et al. (2002) [53] where they observed germination of different plant species in diesel contaminated soil with varying results, they surmised that the delayed seed emergence is as a result of the inhibitory effect of, the fuel fractions in the soil which impedes water and oxygen transfer between the seed and the soil environment thereby hindering germination response. This is further supported by studies done by Ma et al. (2017) [54] on another monocotyledonous plant, Barley seeds, where they identified several physical and chemical factors as being role players in the complex cell signalling that is involved in seed germination but primarily highlighted water imbibition as being the initiating factor for germination and exerting the major influence, therefore the inhibition of water, by the presence of petrol, from entering the coleorhiza in the case of Bermuda grass seeds may have been a plausible reason for its lack of germination and growth during the course of the experiment.

Although Kikuyu grass (monocotyledonous plant) showed marginal growth in petrol contaminated soil, this growth was not of good statistical significance as compared to its growth in cadmium contaminated soil

(96% germination, >80mm shoot length). This is similar to studies done by Okem et al. (2015) [48] using the same plant in cadmium contaminated soil. Previous studied done by Söğüt et al (2005) [55] had also demonstrated the propensity of Pennisetumclandestinum(Kikuyu grass) to accumulate toxic metals from landfill leachates. Similarly, in screening of plants from different mining areas in South America, Pennisetumclandestinumdemonstrated significant metal accumulating abilities with silver [56]. Whilst, studies focused on accumulating abilities, there is little to none existing for mechanism of metal uptake as well as the molecular signaling pertaining to the translocation and tolerance of these metals by the plant. It is also pertinent to note the increased number of germinated seeds of Kikuyu grass in contaminated soil as compared to the control where there was no cadmium and the soil was not sterile, it can be inferred that Kikuyu seems to thrive under chemical stress conditions, although this would require further investigations to validate this assertion.

In comparing the germination of Sunflower and Holly hock (dicotyledonous plants); it was observed that 56% of sunflower germinated in petrol as compared to 14% of Holly hock seeds. In soil contaminated with cadmium, Hollyhock had a significantly higher number of germinated seeds (74%; $\geq$ 45 mm shoot length). It is important to note that the control soil also had less seed germination for Holly hock (<15%) as compared to the seeds planted in cadmium contaminated soil. This also requires further investigation. However, the significant growth of both dicotyledonous plants used in the experiment demonstrates a favourable affinity for these plants towards the biosorption of both pollutants. In a greenhouse study done in Iran to identify the tolerance of sunflower towards its use as a phyto-remediating agent for the clean-up of brownfield sites using growth parameters similar to the current study; the results indicated a tolerance of a maximum of 5000 mg/kg. However, similar to this study as well there was an observable decrease in biomass and height of sunflower in the presence of gasoline in the soil [57]. This study observed very low germination of sunflower seeds on cadmium contaminated soil ($\geq$20%), it is possible that the lack of organic amendments and chelating agents such as EDTA and citric acid as used by previous researchers [49,57] may have further reduced the efficiency of phytoremediation and observable growth of the sunflower plants. The germination and growth of Hollyhock was of significance in cadmium contaminated soil but similar tolerance and biosorption had been observed by Ilhan and Füsun (2018) [51], their research showed 309.3% of cadmium absorption.

Stained cross-sectional images of the stem showed consistently darkened xylem and phloem which are both tissue structures, where the xylem is responsible for the transportation water and dissolved mineral salts to the xylem tissue of leaves; the phloem is responsible for the transportation of food from the leaves of the plant to the cells of the roots. These two transportation structures are clearly demarcated from each other by the cambium. The darkening of these structures is a clear indication of its involvement in the transportation of these pollutants within the plant structures. In a review done by Page and Feller (2015) [58], they highlighted the role played by these two tissues in the re-distribution of micro-nutrients and toxic metals in plants and highlighted the mobility of metals such as nickel, zinc and cadmium and the tendency of the latter to accumulate in meristems.

IV. CONCLUSIONS

The problems caused by environmental pollution calls for the exploitation of environmentally friendly approaches. Accordingly, this study focused on the role of plants in the biosorption of petroleum and cadmium in simulated soil pollution. The observation of seed germination from the four different plants in the presence of these pollutants varied however, it was observed that seed germination was influenced positively or negatively by the type of pollutants used. For example, in seed germination of monocotyledonous plants, Kikuyu grass performed better in cadmium contamination. As for the dicotyledonous plants, Sunflower performed better in petroleum whereas the Hollyhock did better with cadmium contamination. This is an indication that different plant has different capabilities in terms of pollutants that they can remediate. Thus it can be concluded that for effective phytoremediation, the selection of plants based on their capabilities during phytoremediation is of high importance. Further studies are however needed to ascertain structural and morphological correlation in biosorptive efficiency.

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