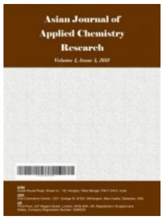

Asian Journal of Applied Chemistry Research

3(1): 1-10, 2019; Article no.AJACR.48785

Potential of *Sorghum bicolor* L. (Moench) and the Effectiveness of Some Organic Amendments in Remediation of Petroleum Oil-Vitiated Soils of an Automobile Repair Workshop in Urbanite Kampala

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJACR/2019/v3i130085
Editor(s):
(1) Dr. Musri Musman, Professor, Department of Chemistry, Syiah Kuala University, Indonesia.
Reviewers:
(1) A. E. Mbachu, Nnamdi Azikiwe University, Nigeria.
(2) Dr. C. O. Nwoko, Federal University of Technology, Nigeria.
(3) Dr. Venkata Sanyasi Seshendra Kumar Karri, GITAM University, India.
Complete Peer review History: http://www.sdiarticle3.com/review-history/48785

Original Research Article

Received 15 February 2019
Accepted 30 April 2019
Published 24 May 2019

ABSTRACT

Aims: To determine the potential of sweet sorghum (*Epuripur 1995*) and the effect of organic biostimulators: NPK fertilizer, cow dung and sewage sludge in remediation of petroleum oil-adulterated soils from a garage in Kampala Metropolis, Uganda.
Place and Duration of Study: The contaminated soils were obtained from New Katanga Boys automobile repair workshop in Wandegeya, Kampala, Uganda. Experiments were conducted

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between September 2018 to November 2018 at Department of Food Processing Technology, College of Agriculture and Environmental Sciences, Makerere University, Kampala, Uganda.
Methodology: 50 kg of petroleum oil-contaminated soils were collected from the garage and divided into 5 kg portions; four portions were potted with four sorghum grains with three subjected to 5% w/w amendment using NPK fertilizer, cow dung and sewage sludge under normal growth conditions for 72 days. Representative soil samples were collected from spots at 0-10 cm and 10-20 cm from the potted soils and subjected to Soxhlet extraction. Growth parameters (leaf surface area, root mass and mass of sorghum heads) of the potted plants were measured.
Results: The sorghum plants grew normally and survived in the petroleum-contaminated soils. Sorghum potted in contaminated soil without any amendment did not flower. Amendment of the vitiated soils with NPK fertilizer, cow dung and sewage sludge biostimulated the phytoremediation capacity of sorghum by 9.1%, 12.5% and 6.3% respectively.
Conclusion: Addition of cow dung to spent-oil contaminated soils could make such soils fully re-established for agricultural activities. Further research should assess the chemical properties of the investigated vitiated soils and the effectiveness of other biostimulants such as vermicompost in biostimulating phytoremediation by *Sorghum bicolor*. The potential of other cereals such as corn, barley, rye and millet in phytoremediation of petroleum-adulterated soils should be investigated.

Keywords: Biostimulator; Epuripur 1995; resource curse; urbanite Kampala; phytoremediation.

1. INTRODUCTION

There has been a peak energy demand globally for diverse domestic and industrial purposes, especially following the invention of internal combustion engines. In Uganda, petroleum and its products utilized entirely for automobiles and thermal plants factor 9.7% to the gross national energy [1]. Uganda, a third world nation is growing steadily with lucrative commercial ties with the Western world. Despite its estimated 800 million barrels of oil discovered in the Albertine Graben in 2006 [2], it is still a ‘‘resource curse’’ that it imports petroleum and petroleum products, used cars and second-hand machines from Japan, United Kingdom, United Arab Emirates, Singapore and South Africa [3,4]. Some of these imports are in compromised mechanical conditions, often requiring maintenance and servicing to enhance their intended performance. This calls for the establishment of automobile repair workshops (garages), which in turn aggravate the risk of soil contamination by spillages of petroleum and petroleum products. Petroleum (hydrocarbon) based products vitiate soil quality as the oil that infiltrates the soil persist for long periods of time [5]. Worse still, the oil suppresses nutrient availability [6,7] and retards water and nutrient absorption by plants [8]. In some cases, the oils accelerate toxic trace metal accumulation in the target soils [9].

The integrity of these petroleum oil-vitiated soils could be reestablished using locally available plants. Several approaches for elimination of petroleum-oil in vitiated soil matrices include bioventing, soil washing, excavation, landfilling, incineration and land farming but usually carry prohibitive costs rendering bioremediation a feasible strategy [10]. Phytoremediation is a nascent eco-friendly and economically credited green environmental strategy for elimination of trace metals [11-13] and other soil contaminants in soils of vitiated qualities [14,15]. Plants utilized in remediation produce hazardous biomass with elevated levels of toxins, restricting their utilization as food and feed. Thus, the choice of plants with demonstrated remediation potential is key in effective phytoremediation.

Sorghum bicolor L. (Moench) (*Epuripur 1995)* is a widely cultivated cereal in Uganda (third after maize and millet) and ranks among the top cultivated and consumed cereals worldwide [16]. It flourishes in nearly all environmental conditions and have excellent phytoremediation potential in adulterated soils [17]. Wastewater contaminated with Cadmium, Lead and Arsenic was used in an experimental irrigation of *S. bicolor* (L.) Moench by Shafiei and associates [18]. They reported that bioconcentration potential of *S. bicolor* is relegated to the priority trace metals and their corresponding concentrations. Phytoremediation of the trace metals in the investigation followed the chemical sequence: Cadmium = Lead > Arsenic whereas tissue accumulation based on dry ash weight was equal for Cadmium and Lead with ions significantly differing in accumulation on dry weight basis. The plant potential to concentrate the trace metals followed a chemical sequence: Cadmium >Lead > Arsenic.

Phytoremediation of Lead contaminated soils by *S. bicolor* was investigated by Gandhi, Sirisha and Asthana [19]. Their results berwayed that *S. bicolor* L. (Moench) is a suited phytoextractor with a translocation factor (TF) less than 1, well higher than the bioaccumulation factor (BCF). At low concentrations, *S. bicolor* remediation was efficient though this diminished at elevated Lead concentrations; chelated assisted techniques employed comparatively in the investigation registered success in reducing the trace metal toxicity, with the physicochemical properties of the soils reducing drastically to WHO permissible limits [19].

Oh et al. [20] assessed the remediation capability of *S. bicolor* and the enhancement effects with microbial inoculation in Lead, Nickel and Copper contaminated soils. Results pointed that sorghum survived the priority trace metals toxicity, and Lead-tolerant fungus inoculation enhanced the growth and phytoremediation of Lead, Nickel and Copper. The phytoextraction potential (evaluated in µg/plant) were respectively 73 for Copper, 410 for Lead and 74 for Nickel whereas 93 (Copper), 590 (Lead) and 120 (Nickel) were recorded following microbial inoculation as an amendment. They recommended sorghum as a promising cereal for phytoremediation of adulterated soils.

Phytoremediation of Chromium metal polluted soils of Ranipet Tanneries was assessed utilizing *S. bicolor* plant as a phytoremediator by Revathi et al. [21]. The impact of the trace metals on the biomass, chlorophyll content and the amendment effect of vermicompost biosolids on *S. bicolor* bioaccumulation efficacy were evaluated. The findings revealed that a significant biomass decrement of the plant was noted with increased trace metal dosing meanwhile inclusion of vermicompost enhanced *S. bicolor* biomass.

Morphophysiological characterization of sweet Sorghum 'M-81E' by Jai et al. [22] revealed that the plant effectively phytoremediated Cadmium metal without any negative growth consequences of the trace metal contamination in the growth media. Hydroponic assessments reported that the biomass of 'M-81E' had no detectable change at 10µM trace metal dosage. Trace metal concentration was elevated in the roots of both germinating and matured plants. Probing histochemical assays with dithizone staining showed that the trace metal was stored primarily in the root stele and haphazardly distributed in the intercellular spaces of the caulicles. Further analytical correlation studies in the caulicles and the leaves revealed that the trace metal exhibited a marked negative correlation with other trace metals: Zinc, Manganese and Iron and a positive correlation with Iron in the plant roots. They concluded that sorghum is a promising candidate for the remediation of Cadmium-adulterated soils.

Cesium (Cs) bioaccumulation properties by two cultivars of *S. bicolor*: Cowly and Nengsi 2 was assessed hydroponically at 50-1000 µmol/L concentration and in soil with spiked metal concentrations of 100 and 400 mg/kg soil by Wang et al. [23]. The plants potted for 100 days had no significant differences in their heights, dry weight and metal bioconcentration. The *S. bicolor* varieties exhibited marked phytoextraction potential of Cs from the adulterated soils with the bioaccumulation and translocation factors greater than 1 in the soil and hydroponic systems respectively. The shoot of *S. bicolor* reportedly removed up to 92% of Cs. The metal at 100µmol/L in solution had the highest BCF and TF indices whereas Cs at lower concentrations were translocated to the plant shoot. Cs at higher concentrations had reduced transfer tendencies from the root to the aerial parts. Plant growth was considerably retarded at concentrations of 400 mg/kg soil and above. The metal was reported in the soil system at 1147, 2473 and 2939 mg/kg in the roots, stems and leaves respectively. On the other hand, the hydroponic system recorded an average metal concentration of 5270 and 4513 mg/kg in the roots and shoots respectively [23].

This study reported the potential of *S. bicolor,* locally known as *Epuripur 1995* in phytoremediation of soils adulterated with petroleum-based oils in an automobile repair workshop. Akin to other nascent technologies in Uganda, plant remediation will be welcomed if and only if its success has been demonstrated with documentation to Environmental authorities like Uganda National Environmental Management Authority (NEMA), Ministry of Energy and Mineral Development (MEMD), Ministry of Finance, Planning and Economic Development (MoFPED) and Economic Policy Research Center (EPRC) for immediate emphasis as a green strategy for reclamation of petroleum oil-contaminated soils. The Upstream Act (cited in [2]) required NEMA to formulate guidelines for extraction, production, transit, storage, treatment and disposal of waste from the petroleum exploitation activities by the end of the year 2017. The results of this study is therefore a resource to the stakeholders involved in the drilling, extraction, transit and storage of the Ugandan crude oil in the Albertine Graben.

2. MATERIALS AND METHODS

2.1 Collection of Samples and Experimental Set Up

Petroleum oil contaminated soils were obtained from New Katanga Boys Automobile Repair Workshop (garage) on Akii Bua road, Wandegeya-Kampala. 50kg of the contaminated soils were collected and divided into five equal portions (5kg each in duplicate). Normal soil was used a positive control. NPK fertilizer was procured from Vap Chemicals Limited**,** 4 Entebbe Road, Kamu Kamu Plaza 7357, Kampala. Cow dung was obtained from Department of Agroprocessing Engineering, Faculty of Engineering, Busitema University, Tororo, Uganda whereas sewage sludge was obtained from Bugolobi sewage treatment plant, Bugolobi, Kampala. Prior to filling into pots, the soil was air dried, ground, homogenized and given the treatments in Table 1. Four sorghum grains (purchased from a local store in Kampala and other grains in the lot previously tested for viability) were potted in each of the five labelled pots, watered accordingly and monitored for 72 days under natural conditions before harvesting.

2.2 Phytoremediation Potential of *Sorghum bicolor* L.

After 72 days, the plants from all the experimental pots were harvested and the petroleum oil in the soils were extracted. A hand auger was used to collect the soil samples from each pot by taking 6 to 10 borings at depths of 0-10 cm and 10-20 cm. Prior to extraction of oil left in the soil samples, the samples were homogenized in a motor to obtain fine mixtures and to remove sticks, pebbles and rock particles. An aliquot (2.0±0.5 g) of the homogenized samples were weighed (Fig. 1) and extracted using the Soxhlet method as per the Brinkman procedures outlined in the US EPA method 3540C [24] with slight modifications in the choice of the solvent, volume, extraction time and size of the extraction flask. The percentage of oil phytoremediated from the soils was computed from the numerical ratio of oil remediated from the soil sample to that in the original soil sample using equation (1).

$$\text{Phytoremediated oil (\%)} = \frac{\mathrm{Mo - Me}}{\mathrm{Mo}} \times 100 \qquad (1)$$

Where *Mo* = mass of oil in the original vitiated soil sample, *Me* = mass of oil still entrained in the soil sample.

Fig. 1. Measurement of vitiated soil weight prior to extraction

The percentage of oil phytoremediated by the sorghum plants potted in control soil is the phytoremediation potential of *Sorghum bicolor.*

2.3 Biostimulation Potential of the Amendment Factors

The biostimulation potential, BP of an amendment factor was calculated using equation (2);

BP = Oil phytoremediated by an amendment factor - Oil phytoremediated by control (2)

Table 1. Experimental set up

Pot number	Potted soil condition	Amendment (5%w/w)
01	Contaminated	NPK fertilizer
02	Contaminated	Sewage sludge
03	Contaminated	Cow dung
04 (Control)	Contaminated	None
05 (Blank)	Normal	None

2.4 Confirmation of the Best Amendment Factor

The sorghum plants were harvested by digging out with care not to break the root branching within the soil. This was made more effective by watering each pot the night before harvest to soften the soil. Growth parameters: mass of roots and heads and leaf surface area of the harvested plants were measured for all the four potted plants on the same day of harvesting to minimize errors due to withering.

3. RESULTS AND STATISTICAL ANALYSIS

Each determination was carried out in quadruplicate and results were reproduced as mean±standard deviations. Data was compared by one-way ANOVA followed by Tukey post hoc test with statistical significance among the means set at *P* = *.01* at www.vassarts.net.

3.1 Germination

All the sorghum grains germinated (Fig. 2).

3.2 Phytoremediation Potential of *Sorghum bicolor*

The percentage of phytoremediated oil from the vitiated soils are given in Table 2. The original soil sample had 0.1700±0.003g of oil per gram of the contaminated soil.

Growth parameters: root mass and the mass of the heads of the harvested plants (Table 3; Fig. 4), leaf surface area (Table 4) were measured in order to confirm the best amendment factor.

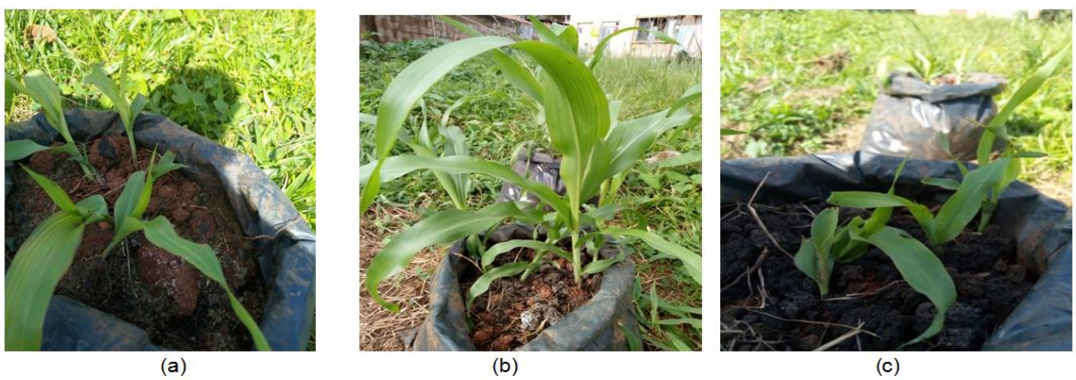

Fig. 2. Experimental potted sorghum plants enhanced with (a) NPK fertilizer, (b) Cow dung, (c) Sewage sludge

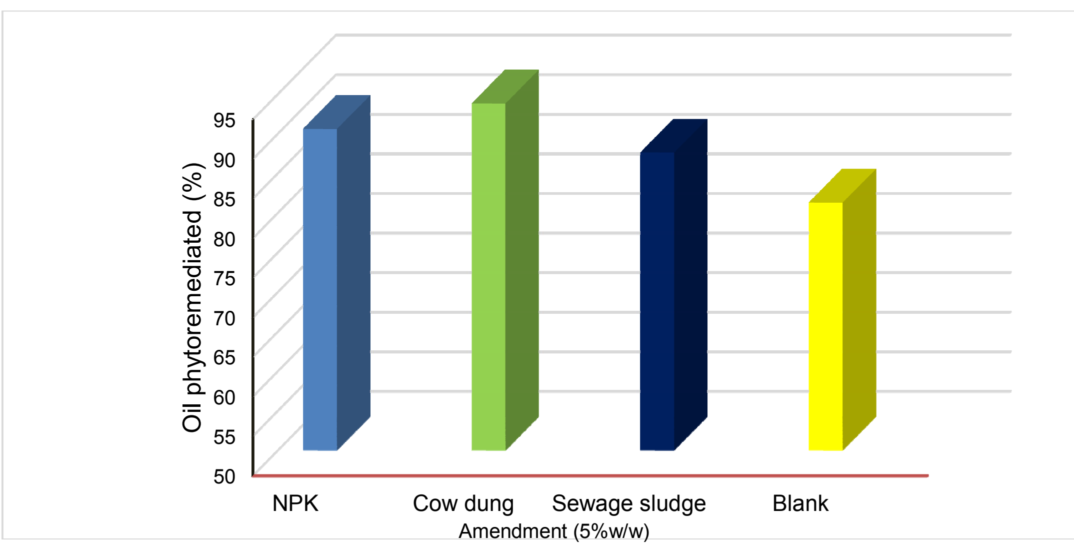

Fig. 3. Effect of biostimulants on the phytoremediation potential of *Sorghum bicolor L.*

Table 2. Phytoremediated oil from the petroleum-contaminated soil samples

Amendment	Oil still entrained in the soil (g)	Oil still entrained in the soil (%)	Oil phytoremediated (%)	Biostimulation potential (%)
NPK fertilizer	0.01632±0.000042	9.60	90.40	9.1
Cow dung	0.01054±0.000085	6.20	**93.80**	**12.5**
Sewage sludge	0.02110±0.000127	12.41	87.59	6.3
Control	0.03179±0.013222	18.70	81.30	N/A

Table 3. Mass of *Sorghum bicolor* L. heads and roots

Amendment	Mass of head (g)	Mass of root (g)
NPK Fertilizer	10.61±0.02[a]	13.60±0.26[f]
Cow dung	5.88±0.04[b]	**15.21±0.05[g]**
Sewage sludge	6.07±0.05[c]	13.02±0.06[h]
Blank	7.30±0.06[d]	10.63±0.05[i]
Control*	0.00±0.00[e]	5.37±0.08[j]

**Did not flower, masses carrying different alphabetical letters in the same column are statistically different (P = .01) as determined by Tukey's HSD test*

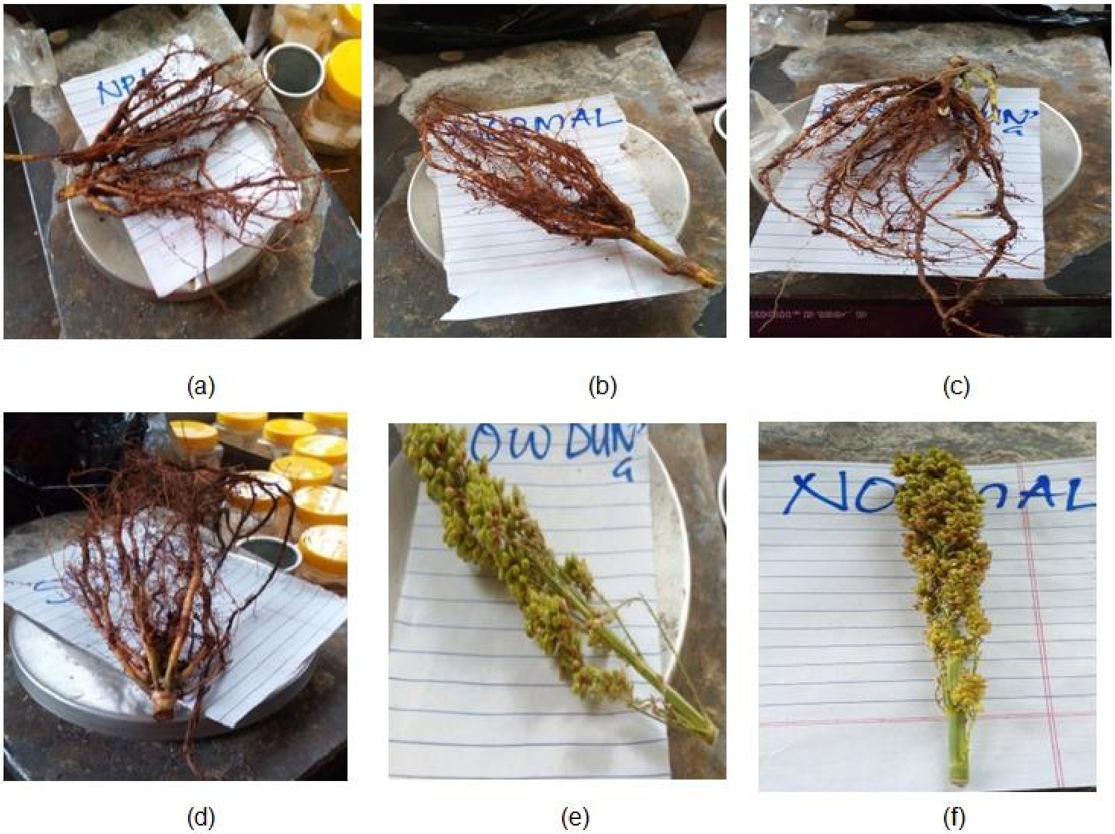

Fig. 4. Sorghum roots and heads from pots of the investigated amendment factors: (a) NPK fertilizer, (b) normal soil, (c) cow dung, (d) sewage sludge, (e) cow dung, (f) normal soil

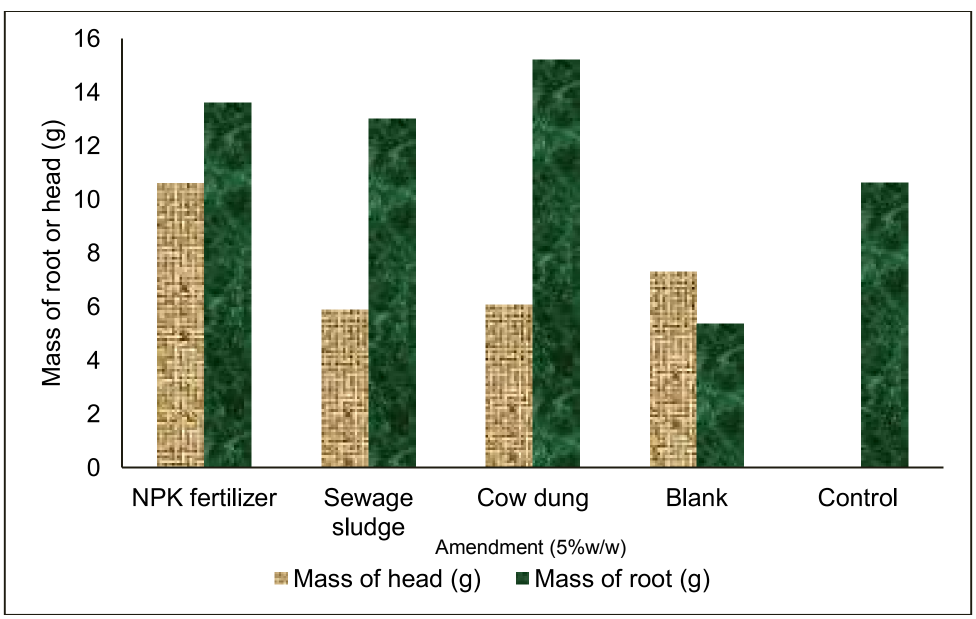

Fig. 5. Effect of the amendments on the mass of the roots and the heads of *S. bicolor*

Table 4. Surface area of *Sorghum bicolor* L. leaves

Amendment	Leaf surface area (cm^2)*
NPK fertilizer	174.93±56.25
Cow dung	**154.67±82.86**
Sewage sludge	220.47±100.12
Blank	67.6±36.79
Control	77.73±32.45

4. DISCUSSION

All the grains potted in this study grew normally. This implied that the growth of the plants are not directly affected by the petroleum contamination of the soil. However, the plants in the contaminated soil without any amendment (control) did not flower (Table 3; Fig. 5). This could be due to growth retardation by the petroleum oil entrained in the potted soil. It is empirically known that petroleum-based oils suppress nutrient availability in soils [6,7] and retards water and nutrient absorption by plants [8]. All these factors are essential for plant growth, flowering and maturity.

Within 72 days, *S. bicolor* without any amendment removed 81.3% of the original oil in the contaminated soil (Table 2; Fig. 3). This demonstrated the potential of sorghum to revitalize the soil integrity after petroleum-oil contamination. This is corroborated by other studies; *S. bicolor L.* and Ryegrass were used to remediate crude oil spill site in Taxas by Gunther et al. [25]. Various species of Kingdom plantae singly or in combination with other amendment factors such as fertilizers and microorganisms have been reported to enhance replenishment of petroleum-contaminated soils [25-27]. Reynolds and Wolf [28] employed Ryegrass (*Lolum multitorum* Lam) to remediate diesel and crude oil-vitiated soils. Pradham et al. [29] conducted a laboratory study with Alfalfa (*Medicago sativa*), switch grass (*Panicum virgatum*) and little bluestem grass (*Selizachyrium scoparium*) that demonstrated potential to remediate total polyaromatic hydrocarbon (PAHs) in vitiated soils at a gas plant.

The results obtained in this investigation showed that the ability of *S. bicolor* to remediate petroleum-contaminated soils can be increased by the amendment factors that were tested. However, all the factors tested have varying efficiencies in boosting phytoremediation potential of sorghum. The reason could be due to difference in their nutrient contents that affect the sorghum plant growth parameters in various ways. Cow dung, among all the tested amendment factors had the highest amendment ability as reflected by the potential of cow dung amended soil sorghum plants to remove up to 93.8% of petroleum oil from the soil (Table 2; Fig. 3). Probably, this could be because cow dung, an excreta of a primary consumer has more nutrients it avail to the sorghum plants that increased their rate of growth and thus the potential to remediate petroleum oil from the soil. Cow dung contain about 3% Nitrogen, 2% phosphorous and 1% Potassium (3-2-1 NPK) [30] with generous amounts of organic matter and other nutrients.

The extensive effect of cow dung was further observed from its effect on the parts of the sorghum plants in comparison to the plants exposed to other amendment factors. Sorghum plants potted in cow dung amended soil recorded the highest mass of roots (15.21±0.05g) (Table 3; Fig. 4). This translated into increased surface area of the roots, which could have enhanced the absorption of the petroleum oil from the contaminated soil. Onwudike [31] in his findings pointed that the fertility of a degraded or highly leached soil can be improved by addition of cow dung singly or in combination with reduced quantity of NPK fertilizer. Further, Njoku et al. [32] reported that there was a general improvement on the growth, dry weight, chlorophyll content, leaf area and pod production of *Glycine max* L. (Merrill) grown in cow dung amended crude oil-polluted soil. It is worth noting that the leaf surface area of sorghum plants grown in cow dung amended soil was the third highest (Table 3) and there was no significant difference (*P =.01*) between the leaf surface areas of sorghum potted in cow dung amended soils and those potted in NPK fertilizer amended soils.

On the other hand, NPK fertilizer, an inorganic fertilizer used commonly for cereals was a better phytoremediation booster than sewage sludge as it biostimulated the phytoremediation potential of *S. bicolor* by 9.1% compared to 6.5% by sewage sludge (Table 2; Fig. 3). This is because although sewage sludge has more nutrients than NPK fertilizer, the latter is majorly constituted by macronutrients (Nitrogen, Potassium and Phosphorous) which are released in a more direct form for easy and fast bioavailability to plants than those nutrients from sludge that are indirect and not easily and immediately absorbed and metabolized by plants.

The growth parameters of potted sorghum plants in normal soil (blank) were far better than the corresponding parameters of the sorghum grown in petroleum-oil contaminated soils without any amendment (control) (Table 2; Fig. 3). The mass of sorghum root from normal soil (blank) was almost twice (10.61±0.05g) greater than for that grown in the contaminated soil without any amendment (control with root mass of 5.37±0.08g) (Table 3; Fig. 5). This illustrates how oil contamination retards the *S. bicolor* growth. Since plant root surface area affects rate of absorption, it greatly determines rate of nutrient uptake which influences growth rate of plants.

5. CONCLUSION

Sorghum bicolor L. (Moench) grew normally and survived in the petroleum-oil contaminated soils. However, plants grown in contaminated soils without any amendment did not flower, thus they suffered the effect of petroleum oil-contamination. Amendment of the adulterated soil with NPK fertilizer, cow dung and sewage sludge biostimulated the phytoremediation potential of *Sorghum bicolor* by 9.1%, 12.5% and 6.3% respectively. Other cereals such as maize, barley, millet that can flourish around the Albertine Graben should be assessed for their phytoremediation efficacy for possible future use in cleaning the soils in the region expected to be heavily polluted when crude oil drilling commence in 2020.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
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