Research Journal of Chemical Sciences ISSN 2231-606X
Vol. **9(3),** 7-16, July **(2019)** *Res. J. Chem. Sci.*

Application of silver nanoparticles during bioremediation of petroleum hydrocarbon-polluted soil by *Eleusine indica* – inhibition or improvement?

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Available online at: www.isca.in, www.isca.me
Received 21st October 2018, revised 27th April 2019, accepted 15th June 2019

Abstract

Nanoparticles (NP) have been associated with numerous aspects of plant applications, including crop production and environmental reclamation. The primary reason is to enhance plants capacity during these applications. The purpose of the research was centred on whether application of biosynthesized silver NP would enhance the bioremediation capacity of Eleusine indica in an oil-polluted soil. Top soil was polluted with spent lubricating oil (obtained as pooled) at 5% w/w. A week later, tillers of Eluesine indica (5-leafed and height 8.3±1.2cm) were transplanted into the oil-polluted soils in experimental bowls. Five weeks after sowing, silver NP were synthesized in the laboratory from silver-trioxonitrate, using aqueous leaf extracts of Azadirachtaindica, Carica papaya, Vernonia amygdalina, Hibiscus sabdariffa, Moringa oleifera. These were immediately applied via foliar spray to each plant at 200ml per plant in divided concentrations of 5, 15 and 30% respectively, from top to bottom. A booster dose was applied after two weeks of initial application. The presence of oil in soil had phytotoxic impact on plant morphological characteristics. There was also significant reduction in reproductive capacity of the plant herein presented as number of panicles per plant. However, with the application of nanoparticles, there was improvement in plant acquisition of panicles. Enhancement in plant reproductive capacity was better with plants sprayed with V. amygdalina-based NP (16panicles), compared to those exposed to A. indica-based NP (11 panicles/plant) and 5 panicles in the plants in oil-polluted soil. The efficiency of remediation of hydrocarbons in the oil-polluted soil by the test plant was enhanced upon application of NP (80.0 – 95.0%) compared to when no NP was applied (65.05%). The study thus accentuates the capacity for NP in enhancing plant survival under environmentally stressed conditions. Further, the enhancement of plant remediative capacity in the oil-polluted soil has also been presented.

Keywords: Ruderals, nanoparticles, bioremediation, Eleusine indica, remediation efficiency.

Introduction

Ruderals abound; they have the capacity to survive were most plant ordinarily would not survive. This characteristic is what is most prominent during selection of plant species for remediation purpose. Among these ruderals is *Eleusineindica* (goose grass). Apart from its prominence in phytoremediation of soils polluted with pesticides[1,2] and heavy metals[3-6], it is also a lawn plant which can be used for horticultural purposes[1,4]. Having also reported the preference of the plant for remediation of petroleum hydrocarbons[3,6,7], the researchers sought to investigate if application of nanoparticles would enhance its phytoremediative capacity or inhibit it.

Nanoparticles exhibit usually physical, chemical and biological activity due to their reduced small sizes[8,9]. Among all the nanoparticles, silver nanoparticles (AgNP), as pointed out by Ahmed *et al*.[10], have gained much interest particularly owing to its distinctive characteristics which include stability, favourable conductivity, capacity for catalysis and antimicrobial significance. However, there is dearth of information regarding its usage in plant enhancement for phytore mediation.

Today nanotechnology is one of the main focuses in sciences with huge applications and implications both for science and engineering. This technology which encompasses the utilization of matter is fast becoming prominent in health care, cosmetics, biomedical, food security, electricity, optics, and environment protection. Manipulation of material substance is on the atomic, molecular and supramolecular scale. However, no application is ever isolated from synthesis. Chemical synthesis of nanoparticles (NP) is usually impeded by a number of shortcomings. To conquer the limitations of chemical synthesis, the emergence of biological synthesis has become a rather viable alternative. This is even more predicted upon the fact that biological methods are simple and less complicated, cost effective and eco-friendly. Most bio-approaches rely on exploitation of plant extracts. In this study, the NPs were synthesized by different plant extracts (*Azadirachtaindica, Carica papaya, Vernoniaamygdalina, Hibiscus sabdariffa,* and *Moringaoleifera*).

Materials and methods

The study was carried out in a marked plot measuring 5 m by 15 m at the mini Botanic Garden, Department of Plant Biology and

Biotechnology, University of Benin, Nigeria (6°23'53"N 5°36'54.1E). Tillers of *Eluesineindica* (5-leafed and height 8.3±1.2 cm) which were previously raised in a nursery, were transplanted only oil-polluted soils in experimental bowls (not perforated). Soils were originally polluted using spent engine oil at 5% w/w.

Five weeks after sowing, silver nanoparticles were synthesized in the laboratory from 1mM silver-trioxonitrate, using aqueous leaf extracts. Fresh leaves of *Azadirachta indica, Carica papaya, Vernonia amygdalina, Hibiscus sabdariffa, Moringa oleifera* were washed, finely cut and macerated, and thereafter placed in a 500ml Erlenmeyer flask. Thereafter 100ml distilled water was added and the suspension boiled for 3 min. 100ml of 1mM aqueous solution silver-trioxonitrate was measured out and added unto 5ml of pure broth. The methods of Shankar and coworkers[11], as modified, were adopted in the biosynthesis of silver nitrate nanoparticles used in the study.

The stock culture prepared was further diluted into 3 concentrations –5, 15, and 30% respectively. These were immediately applied via foliar spray to each plant at 200ml per plant, from top to bottom. A booster dose was applied after two weeks of initial application.

The study was performed in a well-ventilated screen house during the month of May 2018. Each of the experimental bowls was adequately watered whenever there was need at less four times in a week. The Soil Moisture Feel Test was utilized as a measure for ensuring that the soils were adequately moisturized[12]. The experiment was allowed for 3 months. Parameters were scored the moment growth changes began to occur. Plants physical responses to experimental conditions were recorded on periodic basis. Changes in total hydrocarbon content in soil were also determined according to modified methods adopted by the Washington State Department of Ecology[13]. Remediation efficiency as well as contamination factors were also determined[14].

Results and discussion

The physical chemical characteristics of soil before contamination with waste engine oil have been presented on Table-1. Soil pH was 5.27, with an electric conductivity of 301.21µs/cm and total nitrogen of 0.18%. The soil was significantly ferruginous, with 1011.92mg/kg iron content.

As presented on Table-2, morphological characteristics of the plant 6 weeks after application of NP showed minimal differences ($p>0.05$) in plant height compared to the control. However, results from post-hoc statistical data showed application of 15% AN significantly differed from the control by a factor of -30%, thus indicating a reduction. There was a 75% increase in number of leaves per plant upon application of 5% BN. Similar increase in foliage upon nano intervention was recorded in plants exposed to 15% BN. Soil pollution with oil significantly reduced total number of tillers per plant as well as the plant's reproductive capacity, herein assessed as number of panicles ($p<0.05$). Compared to the general control (25 panicles in total), there were 4 panicles in the oil-exposed plants, indicating a 84% reduction as a result of oil contamination. Observably, although oil pollution did not significantly impact on foliage, rooting parameters as well as plant height, its impact on plant reproductive capacity was significant. However, with the application of nanoparticles, there was improvement in plant acquisition of panicles. There were 16 panicles in 5%BN and 11 in 5%AN, compared to 4 in the plants not exposed to NP ($p<0.05$).

The study also showed that the significant reduction in number of roots occasioned by oil pollution was neutralized upon application of 15%BN and 15%ZN. The present study may have shown enhanced rooting characteristics of the test upon exposure to NP, the fact that ZnO NPs inhibited root length of rice has been reported previously[15]. Similarly, alumina-nanoparticles have been reported to suppress root growth of *Triticumaestivum*[16]. Figure-1a and 1b presents a morphological appearance of test plants after termination of experiment.

Table-1: Physical and chemical properties of soil before soil contamination… or before application of treatments.

Parameters	Soil
pH	5.27
Total organic carbon (%)	0.49
Exchangeable acidity (meq/100g soil)	0.22
Electrical conductivity (µs/cm)	301.21
K (meq/100g soil)	1.48
Ca (meq/100g soil)	14.32
Fe (mg/kg)	1011.92
Mg (meq/100g soil)	12.01
Na (meq/100g soil)	10.90
Total Nitrogen (%)	0.18
NO^-_2 (mg/kg)	16.43
NO^-_3 (mg/kg)	30.01
Clay (%)	5.13
Sand (%)	87.81
Silt (%)	7.06

Table-2: Plant characteristics in oil-polluted soil 6 weeks after application of nanoparticles.

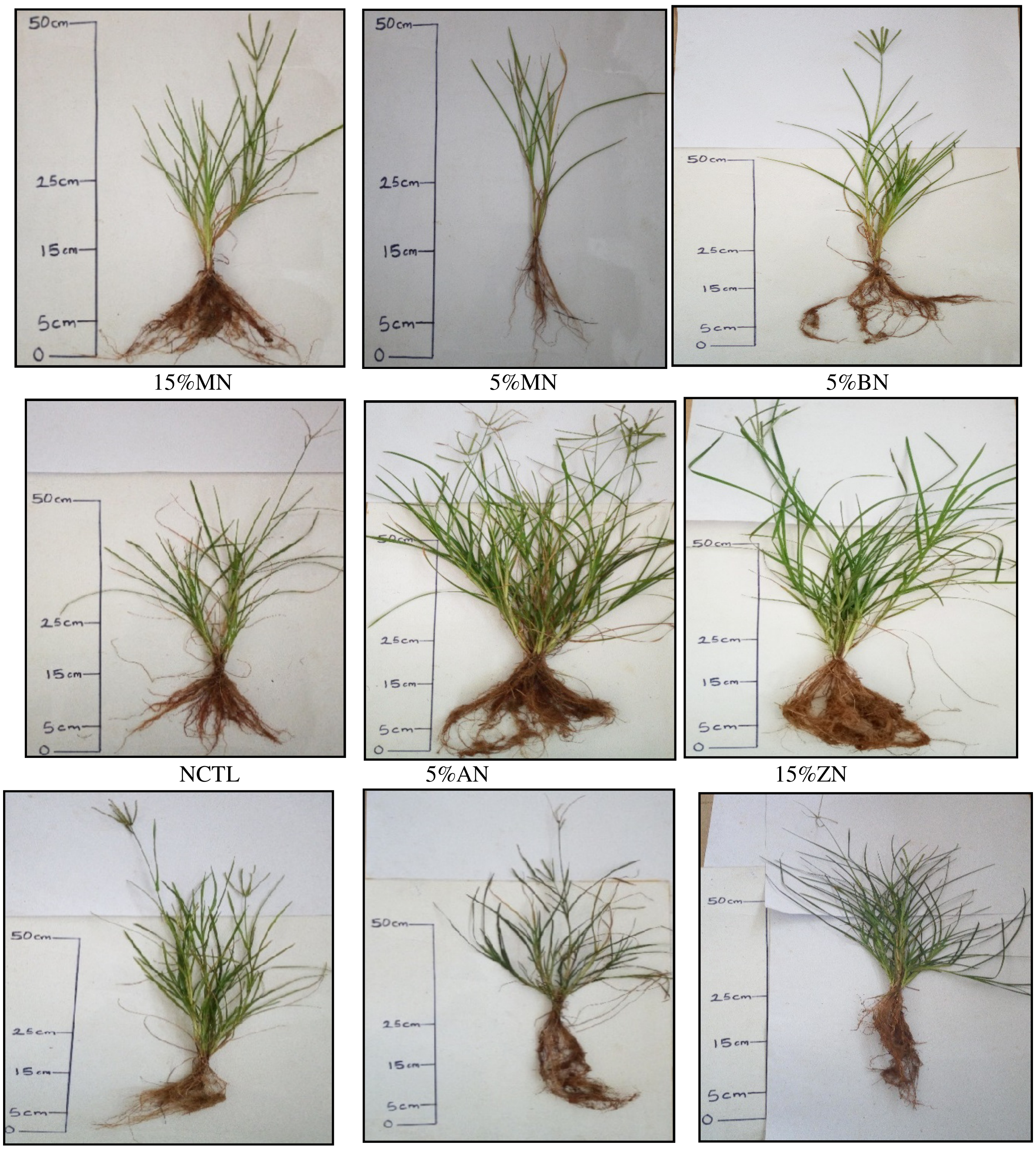
	Plant Height		*No. of Leaves		Leaf Area		Flag leaf length		Internode (cm)		*No. of Tillers		*Total No. of Panicles		*No. of Roots	
	(cm)	%Δ	(No.)	%Δ	(cm^2)	%Δ	(cm)	%Δ	(cm)	%Δ	No.	%Δ	No.	%Δ	No.	%Δ
5%BN	29.3	12.8	63	75	6.78	4.6	20.3	9.3	5.33	45.4	11	-31	16	-36	34	-38
5%MN	33.0	26.9	35	-3	6.89	6.3	19.4	4.4	4.56	22.7	7	-56	6	-76	39	-29
5%ZN	24.7	-5.1	32	-11	5.81	-10.0	19.3	3.9	3.83	4.5	5	-69	7	-72	40	-27
5%CN	21.5	-17.0	24	-33	6.36	-2.7	19.8	6.4	2.57	-30.0	4	-75	4	-84	30	-46
5%AN	33.0	26.9	42	17	7.45	15.1	18.2	-2.2	8.67	136.0	7	-56	11	-56	36	-35
15%BN	27.5	5.8	51	42	5.73	-12.0	17.1	-7.9	4.17	13.6	9	-44	8	-68	53	-4
15%MN	32.7	25.7	30	-17	6.42	-0.9	19.0	1.9	5.87	60.0	5	-69	5	-80	33	-40
15%ZN	26.7	2.5	47	31	6.55	1.2	20.0	7.5	5.33	45.4	9	-44	9	-64	50	-9
15%CN	18.2	-30	28	-22	4.80	-26.0	13.7	-27.0	1.67	-55.0	4	-75	3	-88	28	-49
15%AN	33.0	26.9	23	-36	6.59	1.8	17.6	-5.5	4.67	27.3	4	-75	8	-68	31	-44
30%BN	21.2	-19.0	22	-39	6.25	-3.5	18.8	1.2	4.01	9.1	3	-81	2	-92	29	-47
30%MN	26.1	0	39	8	5.28	-19.0	15.7	-16.0	4.53	23.6	7	-56	5	-80	45	-18
30%ZN	32.2	23.1	37	3	8.63	33.2	24.7	32.6	5.33	45.4	6	-63	6	-76	32	-42
30%CN	24.7	-5.1	23	-36	6.28	-3.1	18.0	-3.2	2.67	-27.0	3	-81	5	-80	35	-36
30%AN	26.1	0.3	22	-39	7.23	11.6	22.3	20.1	2.67	-27.0	4	-75	3	-88	32	-42
NCTL	25.7	-2.7	32	-11	6.31	-2.62	17.9	-3.76	4.31	17.44	8	-50	4	-84	38	-31
GCTL	26.4		36		6.48		18.6		3.67		16		25		55	
LSD(0.05)	8.1	-	12	-	4.03	-	9.4	-	2.06	-	7	-	10	-	13	-
p-value	0.98	-	0.51	-	0.41	-	0.26	-	0.72	-	0.04	-	0.02	-	0.63	-

*Mean has been reduced to nearest whole number. BN nanoparticles from *Vernoniaamygdalina* extract, MN nanoparticles from *Moringa oleifera*, ZN nanoparticles from *Hibiscus sabdarifa,* CN nanoparticles from *Carica papaya*, AN nanoparticles from *Azadirachta indica*, NCTL plant sown in oil-polluted soil but without any nanoparticle intervention, GCTL general control (plant in clean soil, without nano-intervention), SOIL oil-polluted soil without plant. %Δ percentage increase(+) or decrease(-).

Results of reduction in total hydrocarbon contents (THC) of soil at the 11th week have been presented on Table-3 (note: plants were inoculated by NP at week 5). The total hydrocarbon content of soil without plant presence at the 11th week was 9984.9 mg/kg (SOIL). THC for soil with plants was 175.5mg/kg in the unpolluted soil (GCTL) and 3487.7 mg/kg in the polluted soil (NCTL). Results also showed that contamination factor was highest in the soil without plant or nanoparticles (56.9). However, with the application of nanoparticles contamination factor significantly decreased to a range of 3.14 to 12.0 compared to 19.8NCTL. Therefore in terms of efficiency of remediation of hydrocarbons, there was a remediation efficiency of 55.05% in NCTL. This value was significantly surpassed with application of NP to remediations plants (i.e. 78.3% to

91.5%). An attempt was made to bivariate correlated plant parameters exposed to the nanoparticles in an oil-polluted soil and results showed significant bivariate correlation between number of leaves and numbers of tillers (r=0.697, p>0.01) (Table-4). Positive correlation also existed between number of tillers per panicle (r = 0.896, p > 0.01).

The presence of oil in soil has phytotoxic effects. In the present study however, morphological impact of oil, at least at 5%w/w was not significant. However, there was significant reduction in reproductive capacity of the plant herein presented as number of panicles per plant. The application of Np however enhanced plants capacity. This therefore underscores the capacity for NP in enhancing plant survival under environmentally stressed conditions. Further, the enhancement of plant remediative capacity in the oil-polluted soil has also been presented.

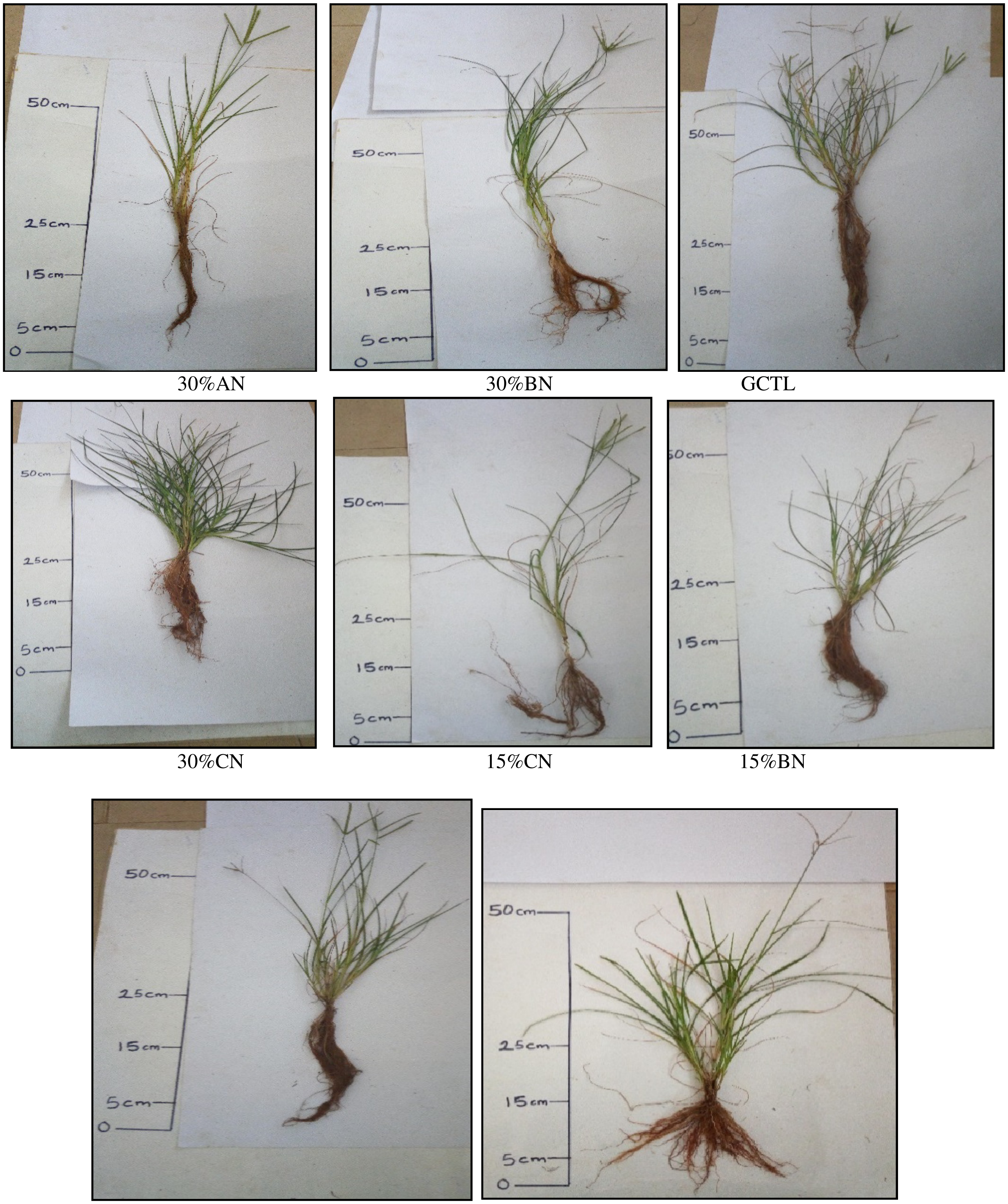


15%MN 5%MN 5%BN

NCTL 5%AN 15%ZN

15%AN 5%ZN 5%MN

Figure-1a: *Eleusine indica* after termination of experiment



30%AN 30%BN GCTL

30%CN 15%CN 15%BN

5%CN 30%ZN

Figure-1b: *Eleusine indica* after termination of experiment.

Figure-2 and Table-5 show the principal component analysis (PCA) of selected plant growth parameters after exposure to nanoparticles as shown below. The PCA presented 2 components or factors among the parameters of interest. On component-1, yield parameters were presented as factors of significant contribution to variability. These included tillering success, panicle no. per plant, additional panicles, panicle per tiller and number of tillers. Component-2 presented flag leaf length, leaf area, no. of leaves, as well as plant height as a group of parameters, majorly foliar parameters, that were also very most likely influenced by exposure to NP in the oil-polluted matrix.

Table-3: Remediation of oil-polluted soil by the test plant upon nano-intervention.

	Total hydrocarbon content (mg/kg)	Contamination factor	Remediation efficiency (%)
GCTL	175.5	NA	NA
SOIL	9984.9	56.9	NA
NCTL	3487.4	19.8	65.05
5%BN	979.6	5.58	90.2
5%MN	2163.3	12.3	78.3
5%ZN	2036.8	11.6	79.6
5%CN	1555.1	8.86	84.4
5%AN	722.5	4.12	92.8
15%BN	1163.3	6.63	88.3
15%MN	857.1	4.88	91.4
15%ZN	1820.4	10.4	81.8
15%CN	1493.9	8.51	85
15%AN	873.5	4.98	91.3
30%BN	939.7	5.35	90.6
30%MN	1579.6	9	84.2
30%ZN	1114.29	6.35	88.8
30%CN	551.0	3.14	94.5
30%AN	726.5	4.14	92.7
LSD(0.05)	421.3	-	-
p-value	0.042	-	-

BN nanoparticles from *Vernonia amygdalina* extract, MN nanoparticles from *Moringa oleifera*, ZN nanoparticles from *Hibiscus sabdarifa,* CN nanoparticles from *Carica papaya*, AN nanoparticles from *Azadirachta indica*, NCTL plant sown in oil-polluted soil but without any nanoparticle intervention, GCTL general control (plant in clean soil, without nano-intervention), SOIL oil-polluted soil without plant.

Table-4: Bivariate correlation of plant growth parameters after exposure to nanoparticles in an oil-polluted soil.

	LVnm	LVwd	STwd	INT	FLlt	TSuc	TLnm	PNTLnm	PNPTnm	adPN	RTnm	PTht	LFar	THC
LVnm	1													
LVwd	-0.151	1												
STwd	0.148	0.031	1											
INT	0.466	0.495*	0.399	1										
FLlt	0.090	-0.271	0.286	0.198	1									
TSuc	0.471*	0.238	0.161	0.353	0.048	1								
TLnm	0.697**	-0.050	-0.017	0.244	0.066	0.555*	1							
PNTLnm	0.383	0.267	0.574*	0.536*	0.501*	0.573*	0.423	1						
PNPTnm	0.503*	0.147	0.042	0.276	0.046	0.763**	0.896**	0.514*	1					
adPN	0.028	0.130	-0.162	-0.099	-0.044	0.402	0.722**	0.272	0.816**	1				
RTnm	0.530*	-0.188	-0.042	0.123	-0.100	0.154	0.768**	0.133	0.570*	0.523*	1			
PTht	0.224	0.553*	0.583*	0.732**	0.314	0.342	0.145	0.566*	0.239	-0.012	0.033	1		
LFar	0.077	0.263	0.282	0.498*	0.847**	0.185	0.074	0.630**	0.156	0.012	-0.177	0.616**	1	
THC	-0.282	0.340	0.259	0.136	-0.058	0.086	-0.233	0.026	-0.026	-0.061	-0.279	0.354	0.126	1

*. Correlation is significant at the 0.05 level (2-tailed).**. Correlation is significant at the 0.01 level (2-tailed). LVnm leaf number; LVwd leaf width; STwd stem width; INT internode; FLlt flag leaf length; TSuc tillering success; TLnm number of tillers; PNTLnm panicles/tiller; PNPTnm number of tiller per plant; adPN number of additional panicle; RTnm number of roots per plant; PTht plant height; LFar leaf area; THC total hydrocarbon content.

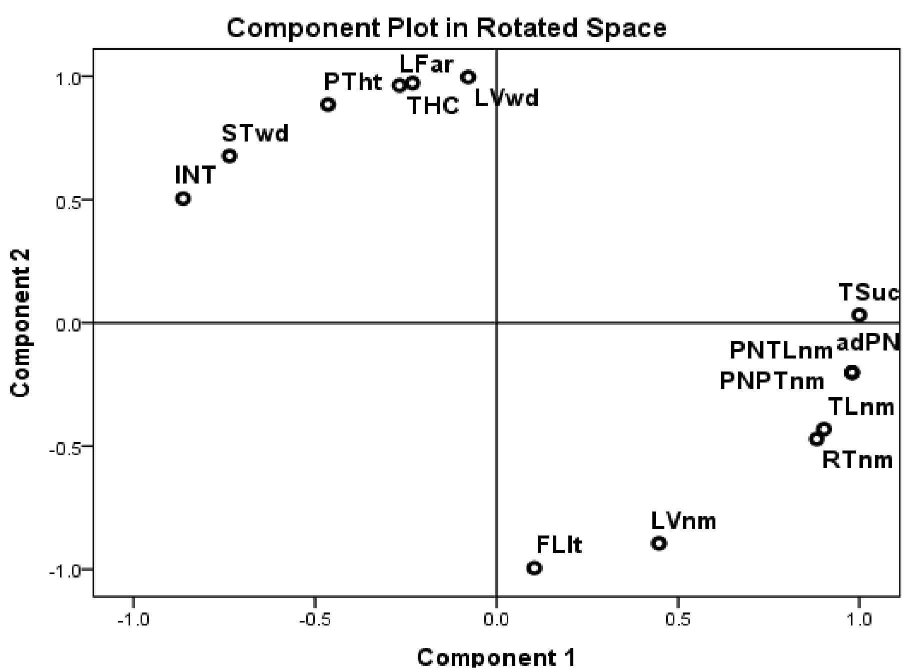


Figure-2: Principal component analyses of selected plant growth parameters after exposure to nanoparticles in an oil-polluted soil. LVnm leaf number; LVwd leaf width; STwd stem width; INT internode; FLlt flag leaf length; TSuc tillering success; TLnm number of tillers; PNTLnm panicles/tiller; PNPTnm number of tiller per plant; adPN number of additional panicle; RTnm number of roots per plant; PTht plant height; LFar leaf area; THC total hydrocarbon content.

The association between selected plant growth parameters and experimental treatments in the oil-polluted soil showed clustering of oil-exposed plants around THC, an indication that their relatedness by hydrocarbon exposure (Figure-3). The general control plants (GCTL) was isolated from the other treatments. This is likely so since the plants were never exposure to oil contamination. There was also a stronger association between plant height and leaf area.

An attempt was made to cluster the individual plant treatments with a view to pairing which ones were more likely similar than the other in terms of the overall presentation of all parameters recorded in the experiment (Figure-4). Two major groups sufficed – NCTL was separated from a combination of all the other experimental units. Recall that NCTL was never exposed to NP. This possibly indicated the exposure to NP enhanced plants performance, particularly as the general control group (GCTL) was paired with a combination of 5%AN, 30%AN, 30%CN, 15%BN, 30%ZN, 15%MN, 30%BN, and 5%BN.

Conclusion

The application of biosynthesized nanoparticles in the reclamation of oil-polluted soil by *Eleusine indica* have been studied. Significant changes in plant morphology was attributed to oil pollution. However, application of NP enhanced plants survivability in the oil-polluted soil. Further, the enhancement of plant remediative capacity in the oil-polluted soil has also been presented.

Table-5: Rotated component matrix of principal component biplot of selected plant growth parameters after exposure to nanoparticles in an oil-polluted soil

	Component-1	Component-2
Tillering success	0.999	0.032
Panicle No. per plant	0.979	-0.202
Additional panicles	0.979	-0.202
Panicle per tiller	0.979	-0.202
Number of tillers	0.902	-0.431
No. of root branches	0.882	-0.471
Internode	-0.864	0.504
Stem width	-0.736	0.677
Leaf width	-0.078	0.997
Flaf leaf length	0.104	-0.995
Leaf area	-0.231	0.973
Total hyd. Content	-0.267	0.964
No. of leaves	0.448	-0.894
Plant height	-0.464	0.886
Extraction Method: Principal Component Analysis. Rotation Method: Varimax with Kaiser Normalization.		

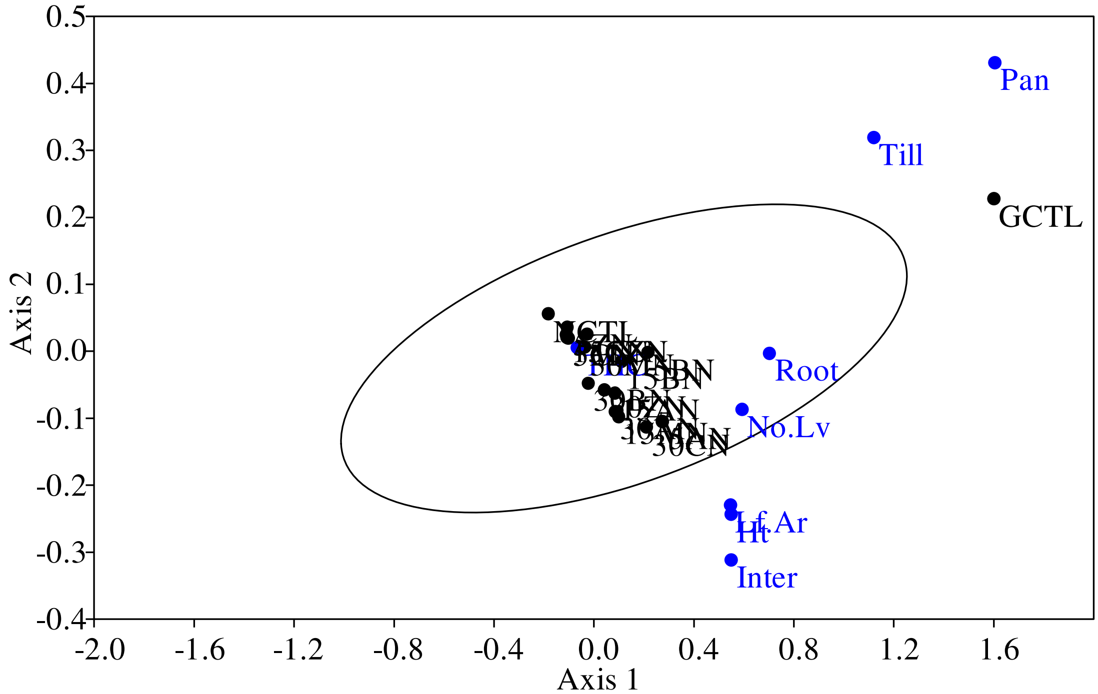


Figure-3: Correspondence analysis of association between selected plant growth parameters and experimental treatments in the oil-polluted soil.

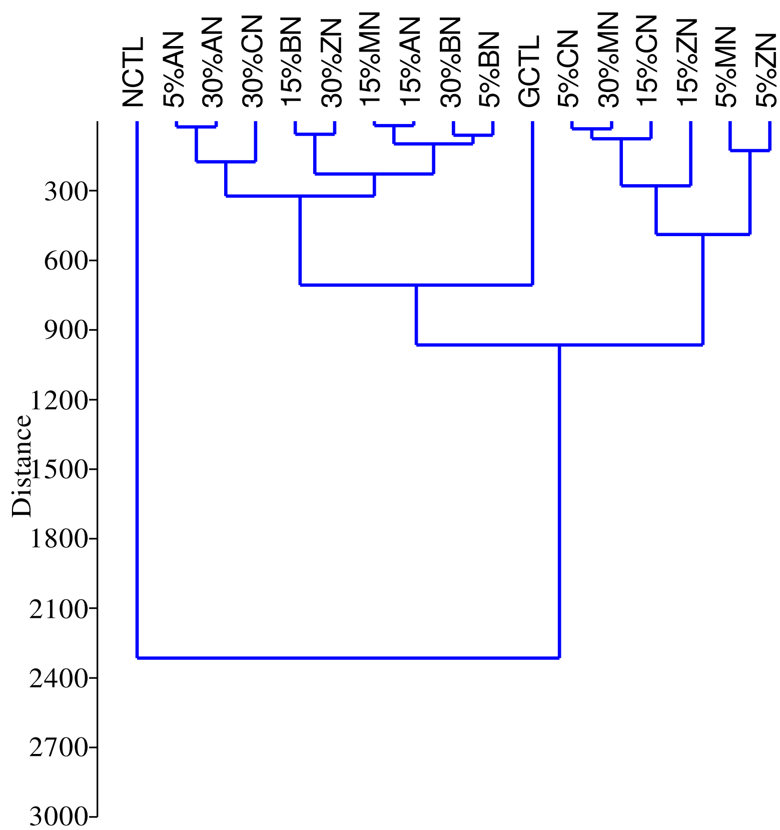


Figure-4: Dendrogram from cluster analysis (Ward's Method) establishing possible paired relatedness of experimental treatments on the basis of experimental outcome.

Acknowledgements

The authors are grateful to Miss Jasmine Pfleger of the University of Technology, Graz. Austria, who was on internship at the EBSR Group Lab during the period of this study. Her contributions are hereby acknowledged. This study was privately funded.

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