Nigerian Journal of Technology (NIJOTECH)
Vol. 39, No. 2, April 2020, pp. **600 - 606**

Print ISSN: 0331-8443, Electronic ISSN: 2467-8821
www.nijotech.com
http://dx.doi.org/10.4314/njt.v39i2.31

REMEDIATION OF ZINC CONTAMINATED SOILS FROM A FARM SETTLEMENT IN NIGERIA

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ABSTRACT
This work is on remediation of zinc concentration (330.0 mg/kg) in soils from farm settlement at Agbabu community in Ondo State of Nigeria to below maximum allowable 300 mg/kg specified for safe agriculture by standards to ensure that farm products from this farm settlement close to area of mining are safe for human beings. Three indigenous organisms: Bacillus subtilis (B. subtilis), Escherichia coli (E. coli) and Proteus mirabilis (P. mirabilis) were engaged for the remediation study. The organisms were isolated and cultured. Optimum weights of the distinct organisms were inoculated in 4g soils each conditioned with optimum values of pH, temperature, stirring frequency and nutrient in thirty-six 50 ml beakers; and experimented for residual zinc ion at times 5, 10, 15, 20 25, 30 and 35 days in triplicate with Atomic Absorption Spectrophotometer. Each organism maintained its performance position from day 5 to day 35. Bacillus subtilis took the lead, seconded by P. mirabilis while E. coli lagged. Removal to safe concentration first occurred at 10 days for B. subtilis, 15 days for P. mirabilis and 20 days for E. coli with respective 292.09 mg/kg, 294.37 mg/kg, and 290. 71 mg/kg residual concentrations. The respective residual concentrations and efficiencies at 35 days were 247.33 mg/kg and 25.06 %; 253.47 mg/kg and 23.20 %; and 267.11 mg/kg and 19.07 %. Two-ways ANOVA at (P < 0.05) showed that a combination of 2 or 3 of the organisms would result in lower residual concentration; and relevant performances at shorter times.

Keywords: *Zinc, contaminated soils, farm settlement, bioremediation*

1. INTRODUCTION

Heavy metals are parts of the natural earth's crust. However, man's activities of diverse nature have boost their concentrations to pollution levels in the environment [1]. Through human activities, dangerous metals have become global problem plaguing many sites [2]; these metals persist due to geoaccumulation and bioaccumulation [3]; and are very difficult to remove [4].

Some of these metals are relevant to the existence and performances of living organisms and other lives at the required concentrations. Above these concentrations benchmarks, they are injurious to lives (humans, plants, and other animals) on planet earth [1].

Zinc (Zn) hazardously affects soils condition, public health, crops qualities and performances at concentration above necessary [5]. Lives health can be in threat through bioaccumulation of heavy metals in the food chain [6]. Zn is not biodegradable, its half-live is long, and can accumulate in body parts [7]. It can be taken-up by plants and find its way in to the human body when the plants are eaten [8]. This is an issue of attention since plants form an essential part of man's diet [9].

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Because of the serious ecological dangers of having these metals in soils, treatment of affected sites is pursuit vigorously with serious drive to understand their hot spots by studying their spatial concentration [10, 11]; and the best treatment alternative.

Treatments housed in physical-chemical methods are effective but with many post-treatment headaches of more toxic products in soil and high cost [12]. Besides, they are incapable of handling certain, low concentrations of metals [13]. This paved way for bioremediation that is still undergoing intensive research to have a more effective cleaning method that is friendly to the environment. Bioremediation has been reported as cost effective, environmentally healthy, and the way forward in treating heavy metals affected lands. Bioremediation, a method of soil cleansing functions on the utilization of mechanisms in-built in microorganisms and plants to remove injurious substances from the ecosystem. Bioremediation with genetically engineered; and indigenous microorganisms have yielded significant and reliable results [5].

In this work, bioremediation of soils from farm settlement in Agbabu community in Ondo State of Nigeria was studied using three indigenous organisms (Bacillus subtilis (B. subtilis), Escherichia coli (E. coli), and Proteus mirabilis (P. mirabilis)). This was aimed at attenuating the soil zinc concentration to below 300 mg/kg specified as the maximum allowable for safe agriculture by standards in [14] to ensure that farm products from these farm settlements close to area of mining are safe for human beings.

2. MATERIALS AND METHODS

2.1 Materials, Nutrients and Reagents

These include soil sample from Agbabu community, MacConkry agar, magnetic stirrer, hydrogen peroxide, measuring cylinder, safranin, refrigerator, simon citrate ager, inoculating nidles, Kovac's reagent, incubator, triple sugar iron agar, microscope, sodium hydroxide, conical flasks, nitric acid, beakers, hydrochloric acid, wire loops, Lugo's iodine, pipettes, oxidase reagent, cotton wool, methylene blue, autoclave, peptone water, petri dishes, ethanol, filter paper, perchloric acid, MacCartney bottles, sulphuric acids, hot plate, peptone water, atomic absorption spectrophotometer and crystal violent.

2.2 Organisms Acquisition

At a microbiology laboratory belonging to Delta State University, Nigeria; microbiology analysis was conducted on the soils to acquire indigenous microorganisms.

Aliquot from serial dilution was introduced into petri dishes, covered with MacConkey agar [15], and incubated for 24 hours at 37^{o}C [16]. Developed Colonies were recognized after they were sub cultured [17, 16].

2.3 Optimum factors Acquisition

Vital factors have been discovered to have significant influence on bioremediation process and rate [18, 19]. The immense scientific significance of these factors at their optimal levels requires that they be carefully studied, screened and selected for a particular bioremediation study

Adopting the batch method in [20], pH values of 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10; temperature values of 10, 20, 30, 40, 50, and 60^{O}C; nutrient dosage of 2, 4, 6, 8, 10, and 12 ml; organisms' weights of 1, 2, 3, 4, 5, and 6g; and stirring frequencies of 0, 1, 2, 3, 4, 5 and 6 per week (pw) were respectively and distinctly introduced into 4g in thirty-four 50 ml beakers and inoculated with the different organisms. The soils samples separated from the organisms were tested for depletion in metal content on the 14th day with Atomic Absorption Spectrophotometer (AAS).

2.4 Ion Removal

Applying the method in [20], the optimum weights of the distinct organisms were inoculated into 4g soils each conditioned with optimum values of pH, temperature, stirring frequency and nutrient in thirty-six 50 ml beakers and experimented for residual zinc ion at times 5, 10, 15, 20, 25, 30 and 35 days in triplicate with AAS.

The concentration removed with time, removal efficiency, and concentration removed at equilibrium were calculated from Equations (1), (2) and (3) [21, 22].

$$q_t = \frac{(C_o - C_t)}{m}.V \quad (1)$$

$$\text{Efficiency } (\varepsilon) = \frac{(C_o - C_f)}{C_o}.100 \quad (2)$$

$$q_e = \frac{(C_o - C_e)}{m}.V \quad (3)$$

Where V is volume of soil used, C_e is equilibrium concentration, C_o is initial concentration, m is the mass of organism, C_t is the residual concentration per time, q_e removal at equilibrium, C_f is the final residual concentration, and q_t is removal with time.

Two-ways (ANOVA) at ($P < 0.05$) conducted with Microsoft Excel, 2016 version was engaged to determine significant variation in removal with organisms and significant variation in removal with time.

3. RESULTS AND DISCUSSION

3.1 Organisms and Optimum Factors

The microbiology experiments revealed B. subtilis, E.coli and P. mirabilis from developed colony of 2.8 x 10^5 with respective biochemical properties of (positive, negative, positive, negative, positive, positive, positive and negative); (negative, negative, positive, negative, negative, positive, negative and negative); and (positive, negative, negative, negative, positive, positive, positive and positive) catalase, citrate, oxidase, indole, glucose, sucrose, motility and lactose analysis.

Significant determinants of effective bioremediation were carefully studied to acquire their optimum values for optimum bioremediation [20]. These include pH, stirring frequency, temperature, organisms' masses, and nutrient dosage.

pH affects the negative charges on cells and the chemistry cell wall; and the metals physiochemistry [23, 24] thus influencing bioremediation. This makes pH a pivotal, critical influence of bioremediation [24]. pH (1, 2, 3, 4, 5, 6, 7, 8, 9 and 10) influence on the ion removal is shown in Figure 1. Optimum values was 8 for the use of B. subtilis; 5 for P. mirabilis and E. coli at respective minimum concentrations of 251.33 mg/kg, 270.24 mg/kg and 261.13 mg/kg remaining in soils.

Temperature, an indicator of heat magnitude supplied to the process is a major determinant of organisms' performances [19]. Its variation influences the process significantly [25].

The influences of the tested temperature degrees are shown in Figure 2 displaying an optimum degree of 30^oC for the organisms. The respective minimum concentration at this optimum degree where 246.15 mg/kg for the use of B. subtilis; 254.29 mg/kg for the use of P. mirabilis; and 257.93 mg/kg for the use of E. coli.

The supply of requisite nutrient is very essential for the stimulation of the indigenous microorganisms for effective performance [26]. Biostimulation by nutrient supply increases the number of organisms through rapid growth and replication, and ultimately increases bioremediation rate [27]. Influence of nutrient dosage of 2, 4, 6, 8, 10 and 12 ml on the organism performances is shown in Figure 3 displaying an optimum nutrient dosage of 8 ml. The influence was in the decreasing order of 8 ml, 6 ml, 10 ml, 4 ml, 12 ml and 2 ml for the use of B. subtilis; 8 ml, 10 ml, 6 ml, 12 ml, 4 ml and 2 ml. for the use of P. mirabilis; 8 ml, 6 ml, 10 ml, 12 ml, 4 ml and 2 ml for the use of E. coli. The minimum concentrations at the optimum nutrient dosage is 250.45 mg/kg for removal by B. subtilis, 270.24 mg/kg for removal by P. mirabilis, and 261.43 mg/kg for removal by E. coli. The organisms' population used in bioremediation bears direct relationship with the collective weight of the organisms brought in contact with contaminated medium. This makes it very vital to engage the optimum weight of organism in bioremediation study. Figure 4 shows the resultant influence of 2, 3, 4, 5 and 6 grams of the respective organisms on the process with the optimum weight of 5g for the respective organisms at the respective minimum concentrations of 248.15 mg/kg for B. subtilis; 268.24 mg/kg for P. mirabilis; and 289.23 mg/kg for E. coli.

The influences of the weights of the distinct organisms were in the decreasing order of 5g, 4g, 3g, 6g, 2g and 1g for removal by B. subtilis; 5g, 4g, 3g, 6g, 2g and 1g for removal by P. mirabilis; and 5g, 4g, 3g, 6g, 2g and 1g for removal by E. coli. Oxygen diffusivity promoted by soil stirring is another essential influencer of bioremediation [18]. Stirring makes available oxygen for microorganism's aerobic activities. Figure 5 shows the influences of stirring frequencies on the organisms' performances. The study showed 5pw at 120 rpm for P. mirabilis; and 5pw at 150 rpm for B. subtilis and E. coli as the optimum stirring frequencies. These values were recognized at the respective residual concentrations of 250.58 mg/kg for B. subtilis; 270.96 mg/kg for P. mirabilis; and 261.06 mg/kg for E. coli.

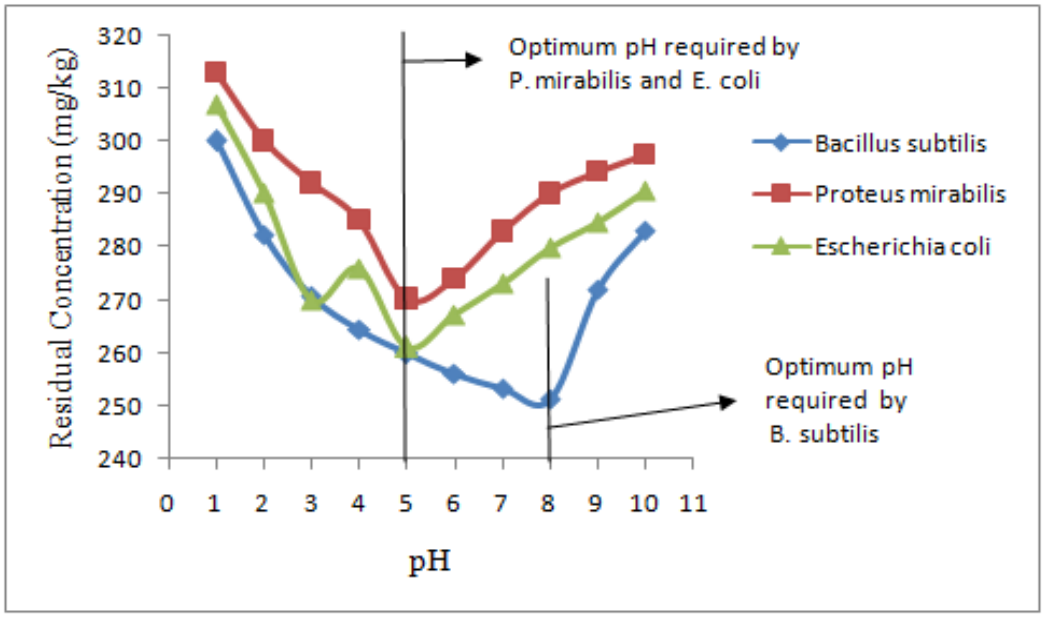

Figure 1: pH and Removal

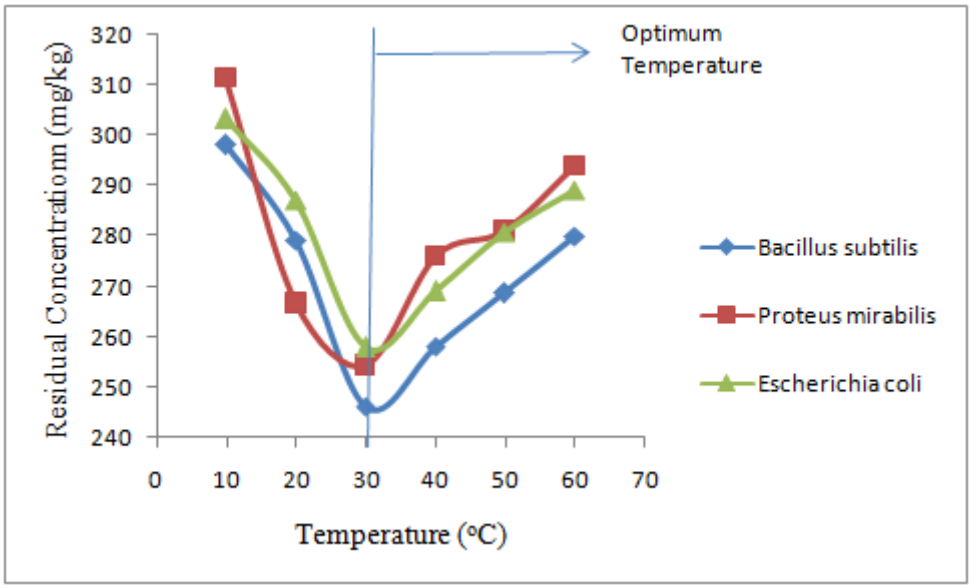

Figure 2: Temperature and Removal

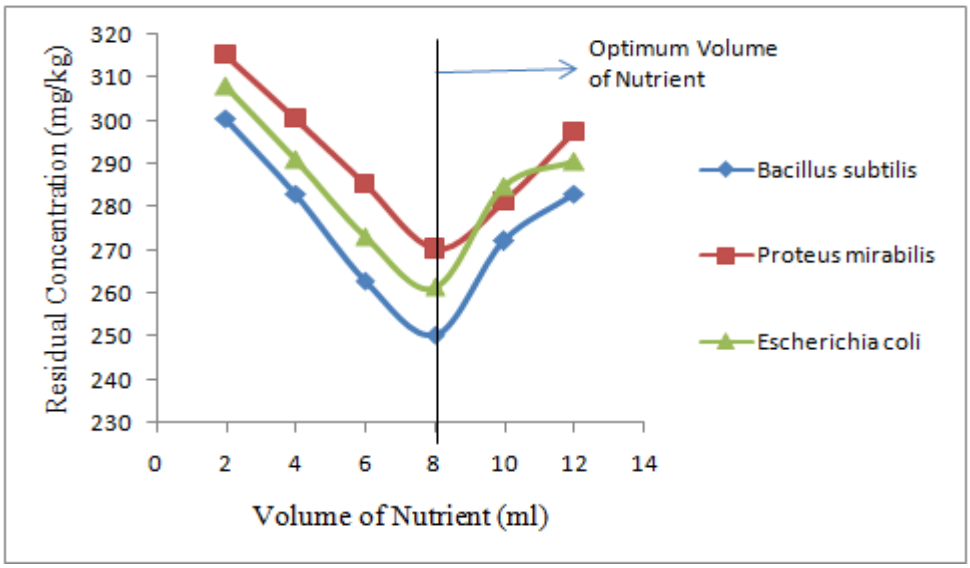

Figure 3: Nutrient Volume and Removal

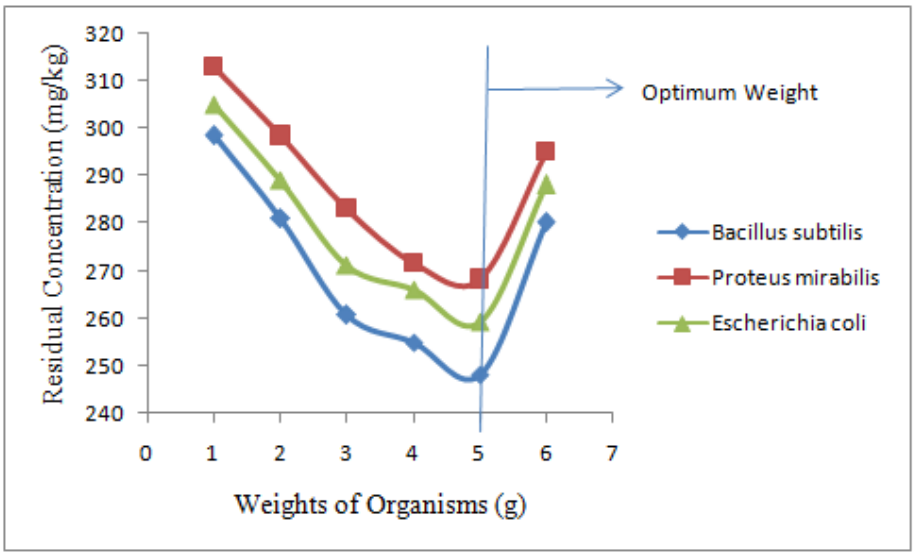

Figure 4: Organisms' Weights and Removal

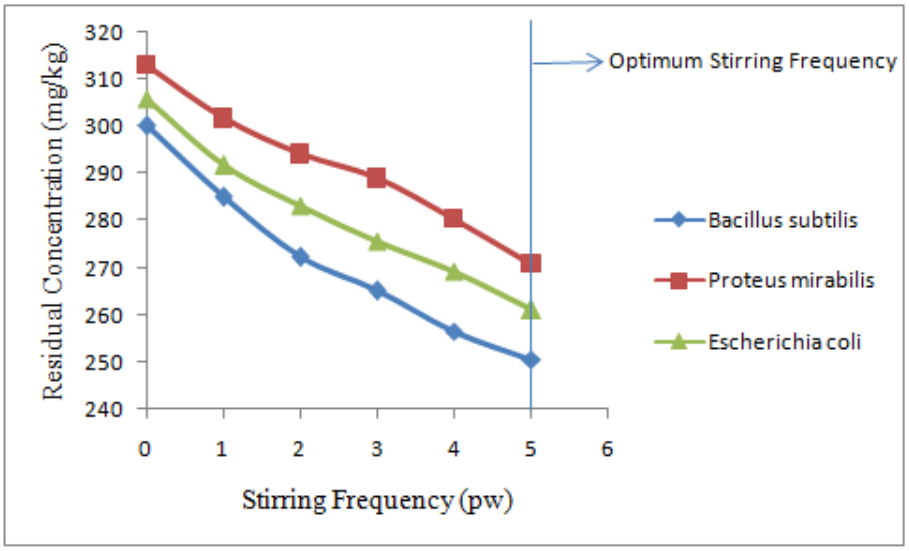

Figure 5: Stirring Frequency and Removal

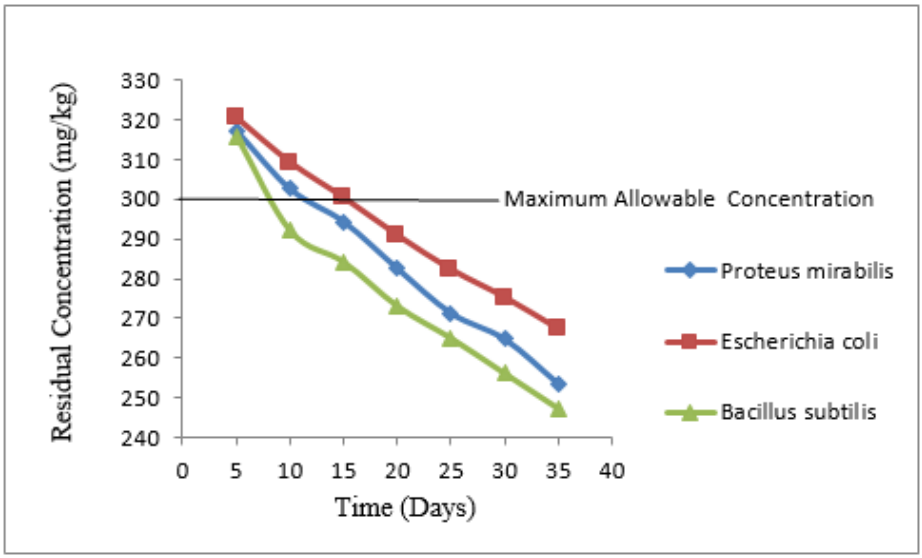

Figure 6: Comparative Impacts of the Organisms

3.2 Comparative Impacts of the Organisms

The metal removal was studied with the optimal values of factors for 5, 10, 15, 20, 25, 30, 35 days taking the maximum allowable concentrations of 300 mg/kg by standards in [14] as the reference for performance rating of the organisms to correct the soil initial concentration of 330.04 mg/kg.

Each organism maintained its performance position from day 5 to day 35 as shown in Figure 6. Bacillus subtilis took the lead, seconded by P. mirabilis while E. coli lagged behind them. The different organisms showed abilities for controlling zinc pollution in the soil-as they were able to bring the initial concentration to below the maximum allowable concentration. This control by the organisms was achieved at different days. It occurred at day 10 for B. subtilis, day 15 for P. mirabilis and day 20 for E. coli.

Removal by B. subtilis on day 10 was at efficiency of 11.50 % and residual concentration of 292.09 mg/kg. The control with P. mirabilis at time 15 days was at efficiency of 10.79 % and residual concentration of 294.37 mg/kg. E. coli, having the least strength for this metal removal brought the pollution under control at time 20 days with an efficiency of 11.92 % and a residual concentration of 290. 71 mg/kg.

The order of removal strength remain constant with time. B. subtilis remained the best and showed a removal efficiency of 25.06% at a residual concentration of 247.33 mg/kg at 35 days; P. mirabilis, the next in removal strength showed an efficiency of 23.20 % and residual concentration of 253.47 mg/kg at 35 days; and the least in performance, which is E. coli, showed an efficiency of 19.07 % with a residual concentration of 267.11 mg/kg at 35 days.

Significant difference at ($P < 0.05$) in the residual concentrations effected by the different organisms showed that a combination of 2 or 3 of the organisms would result in lower residual concentration. Relevant performance was shown possible at shorter times by the ANOVA at ($P < 0.05$). This was reflected by the significant difference in the residual concentrations with respect to time

4. CONCLUSION

This work is on bioremediation of soils from farm settlement at Agbabu community in Ondo State of Nigeria using three indigenous organisms: Bacillus subtilis, Escherichia coli and Proteus mirabilis.

Removal to safe concentration first occurred at different days -10 days for B. subtilis, 15 days for P. mirabilis and 20 days for E. coli with 292.09 mg/kg, 294.37 mg/kg, and 290. 71 mg/kg residual concentrations respectively

At time 35 days, B. subtilis showed a removal efficiency of 25.06 % and residual concentration of 247.33 mg/kg; P. mirabilis showed an efficiency of 23.20 % and residual concentration of 253.47 mg/kg; and E. coli showed an efficiency of 19.07 % and residual concentration of 267.11 mg/kg.

Two-ways ANOVA at ($P < 0.05$) showed that a combination of 2 or 3 of the organisms would result in lower residual concentration; and relevant performance was shown possible at shorter times

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