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Evaluating Polycyclic Aromatic Hydrocarbon Levels and Toxic Impact of Hairdressing Salon Effluent on Soil Nitrifying Bacteria

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Abstract

The recent dramatic increase in the amount of toxic organic waste generation from salons has necessitated researchers to understand the composition of the effluent and its impact on ecosystem functioning. This research focused on the assessment of polycyclic aromatic hydrocarbon (PAH) levels and toxic impact of hairdressing salon effluent on soil nitrifying bacteria. Hair dressing salon effluent obtained from different hair dressing salons in Umuagwo village, Imo state was amended to loamy soil sample in a 25liter Jerrican. After 30 days of pollution, four soil sample categories: Topsoil (0-5cm), Midsoil (12-17cm) and Subsoil (25-28cm) depths, and a non-polluted soil sample, which served as the control, were collected. The PAH contents were determined as well as the microbial load. Further, *Nitrobacter* sp. and *Nitrosomonas* sp. were isolated and used to carryout nitrifying bacterial toxicity analysis. The results show that the following PAHs were present in the sample: 51.03ng/g Biphenyl, 15.01ng/g Benzo[a]pyrene, 31.32ng/g Anthracene, 6.42ng/g Phenanthrene and 5.02ng/g Naphthalene. Coronene and Fluorene were below the limits of detection. However, only 1.90ng/g of Biphenyl was detected in the control garden soil. The total heterotrophic bacteria count in the composted soil had counts ranging from 1.0 x 10^4±1.10 to 2.0 x 10^4±2.10cfu/g while the control soil sample had higher counts ranging from 2.0 x 10^4±0.20 to 4.0 x10^4±0.11cfu/g. The toxicity analysis showed a decline in the heterotrophic bacterial count and both of the nitrifying bacteria were affected in the same manner. The results implies that a constant release of hairdressing salon effluent into the soil poses serious threat to the growth and activities of nitrifying bacterial in the soil. This can affect ecosystem function by reducing soil fertility and crop yield.

Keywords: Nitrifying Bacteria, Hairdressing Salon Effluent, Polycyclic Aromatic Hydrocarbon, Toxicity

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1. Introduction

The continuous trend towards the manufacture of novel hair products and formulation of new beauty products to satisfy the demands of the growing populace causes environmental pollution [1]. Some common hair beauty products that might be found in hair dressing salon effluent include: Shampoo, Conditioner, Hair dye, Hair bleach, hair gel, mousse, hairspray, pomade, hair wax, hair masks, serums, leave-in conditioners, Hair perm solutions, relaxers, Hair glazes, glosses, hair colour, straightening creams or serums, volumizing mousse or spray, Hair oils, Hair serums: protein treatments or keratin treatments [2]. Organic chemicals like ammonium can be present in salon effluent due to various hairdressing processes and products. Excess ammonium thioglycolate may be rinsed off the hair, which is often found in hair dye and hair bleach formulations. Ammonium compounds are also present in many shampoos and hair cleansers as surfactants. These compounds help to lift dirt and oil from the hair and scalp. After a chemical treatment like perm, neutralizers, which help to restore the hair's pH balance often, contain ammonium compounds. These ammonium compounds from residues from cleaning activities can end up in salon effluent [3-4].

Polycyclic aromatic hydrocarbons (PAHs) in hairdressing salon effluent can come from several sources such as dyes, shampoos, conditioners, and styling gels contain various chemicals, including PAHs, which can be released into the effluent during the hairdressing process [5] PAHs present in hairdressing salon effluent can elicit a huge toxic effect on nitrifying bacteria in soil [5]. Nitrifying bacteria play important role in the nitrogen cycle by converting ammonia (NH_3) into nitrite (NO_2^-) and then into

nitrate (NO_3^-). PAHs blocks the metabolic activity of nitrifying bacteria by altering the cellular respiration and enzyme function [6]. This can interrupt the nitrification process, thereby reducing the conversion rates of ammonia to nitrite and nitrate [7]. PAHs have the ability to cause cellular damage in nitrifying bacteria through mechanisms such as oxidative stress and lipid peroxidation. Exposure to PAHs could cause the generation of reactive oxygen species (ROS), which can destroy cell membranes, proteins, and DNA [8]. High concentrations of PAHs in hairdressing salon effluent can damage the growth and reproduction of nitrifying bacteria. Chronic exposure to PAHs could cause a decreased cell viability, reduced population densities, and altered reproductive capacity in nitrifying bacterial communities [9].

PAH contamination can destroy the composition and diversity of nitrifying bacterial communities in soil. A good number of nitrifying bacteria may likely be more sensitive to PAHs than others, creating a shift in community structure and possible loss of key functional groups involved in nitrification [3]. The toxic impact of PAHs on nitrifying bacteria can bring about decreased rates of ammonia oxidation and nitrite oxidation, thereby influencing the whole nitrogen transformation processes in soil. This can result to accumulation of ammonia and nitrite, altering nitrogen cycling and nutrient availability for plants [10]. PAHs may combine together with other pollutants present in hairdressing salon effluent to intensify their toxic impact on nitrifying bacteria. Prolonged exposure to PAHs can result to long-term effects on soil health and fertility by altering nitrogen cycling and nutrient dynamics. Altered nitrification can create nutrient imbalance, low plant productivity, and degradation of soil quality in general [4]. The presences and consequences of PAHs in hairdressing salon effluent on nitrifying bacteria is of great danger to soil fertility, nutrient cycling, and ecosystem functioning. Successful mitigation measures towards reducing PAH contamination and alleviating its impact on nitrifying bacteria are necessary for ensuring healthy soil ecosystems [11]. Therefore, this study assessed the toxic effects of polycyclic aromatic hydrocarbons in hairdressing salon effluent on soil nitrifying bacteria.

2. Materials and Methods

2.1. Material sources and study location

The hair dressing salon effluent was collected from different hair dressing salons in Umuagwo village, Imo state and thereafter mixed together in a Jerrican. Loamy soil samples were obtained from a farmland in the University of Agriculture and Environmental Sciences Umuagwo, Owerri, Imo State. Soil sample collections were done with a standard soil auger (Gilson, U.S). The experiment was set up in microbiology laboratory, University of Agriculture and Environmental Sciences Umuagwo, Owerri, Imo State.

2.2. Experimental set-up

The mixed soil sample was sieved with a mechanical sieve (16.00mm, ASTM-Endecott, U.S). A 25-liter Jerrican with a height of about 30cm was cut open at the top and bottom and filled with the soil sample up to the level of about 28cm. Thereafter, 4 liters of the effluent was poured on the soil sample in the jerrican every day for 30 days. The three soil categories 500g each (Topsoil: 0-5cm dip, the Mid-soil:13-16cm dip and the Sub-soil: 25-28cm dip) were collected from the jerrican after the pollution exercise [5]. 500g of non-polluted soil sample was also collected which served as the control experiment. The four soil sample categories were air dried and taken to the laboratory for the enumeration of heterotrophic bacterial count and gas chromatography analysis.

2.3. Isolation and Enumeration of Heterotrophic Bacteria

A total viable heterotrophic bacterial count was determined using the streak plate technique. Each of the soil sample category was serially diluted up to 10^{-5}-fold dilution. 0.1 ml of diluent was inoculated into duplicate plates of nutrient agar plates and incubated at 37^{o}C for 7 days. This was done for the top soil, mid soil, sub soil and control soil sample. Bacterial counts were recorded every day for 7 days. The nitrifying bacteria: *Nitrobacter* sp. and *Nitrosomonas* sp. were isolated from soil sample and confirmed according to the method of [12]. The *Nitrosomonas* sp. isolate was transferred into a bijou bottle containing 20ml peptone water while *Nitrobacter* sp. isolate was also transferred into another bijou bottle containing 20ml peptone water and both were allowed to stand for 60 seconds to create Nitrosomonas and Nitrobacter liquid suspension. They were kept in the incubator at room temperature until further use.

2.4. Determination of Nitrosomonas and Nitrobacter growth and Toxicity Test

For *Nitrosomonas* set up, hair dressing salon effluents volumes of 100, 200, 300, 400 and 500ml were poured each into a 250ml conical flask. Ten mil of the already prepared *Nitrosomonas* liquid suspension was inoculated into the five-hair dressing salon effluent volumes. A control experiment was set up of the same effluent volumes of 100, 200, 300, 400 and 500ml in a conical flask amended with ten mill peptone water respectively [2]. After an hour, one mil of each of the suspension collected from each of the flask was inoculated into duplicate plates of winograsky agar. Thereafter, they were incubated at room temperature (28+/-2°C) for 24 hours. This was repeated for 2-, 3- and 4-hours interval. After incubation, Nitrosomonas count was recorded. The same procedure was repeated for *Nitrobacter* set up. After incubation, Nitrobacter count was recorded. The percentage inhibitions of *Nitrosomonas* sp. and *Nitrobacter* sp. were calculated using the formula below [2].

$$(\%)\ \text{Inhibition} = \frac{\text{N (control) --- N (sample) X 100}}{\text{N (control)}}$$

N (control) = Number of colonies (cfu/ml) from the control sample.

N (sample) = Number of colonies (cfu/ml) from the effluent samples.

The LC_{50} calculated using the formula below.

Equation (1): $Y = 1.485x + 1.466$

For LC_{50} calculation obtained from a regression line graph, the value for Y in the equations above is the 50% value of the total bacterial inhibition. Each of these values were substituted for Y in the equations and the value gotten for X became our LC_{50} values [1-2].

2.5. Determination of Metabolite

For nitrite production, hair dressing salon effluents volumes of 20, 40, 60, 80 and 100ml were poured each into a 250ml conical flask. The hair dressing salon effluent contains

10% ammonium. 10ml of the already prepared *Nitrosomonas* liquid suspension was inoculated into the five-hair dressing salon effluent volumes. A control experiment was set up of the same effluent volumes of 20, 40, 60, 80 and 100ml in a conical flask amended with 10ml peptone water respectively [2]. These were monitored from 1 to 4hours for nitrite production using a visible spectrophotometry (JOD-778.000, England). After the nitrite production, the same set up was used for nitrite utilization experiment because we used the already produced nitrite concentrations of (51.21, 47.05, 31.22 and 28.65%) for the experiment without supplementation of nitrite. Having the hair dressing salon effluents volumes of 15, 35, 55, 75 and 95ml already. The volumes of the produced nitrite/effluent suspension reduced because 5ml each have been used up for the spectrophotometry analysis.10ml of the already prepared *Nitrobacter* liquid suspension was inoculated into the five-hair dressing salon effluent volumes. A control experiment was set up of the same effluent volumes of 15, 35, 55, 75 and 95ml in a conical flask amended with 10ml peptone water respectively [13]. These were monitored from 1 to 4hours for nitrate concentration using a visible spectrophotometry (JOD-778.000, England) [14].

The EC_{50} calculated using the formula below.

Equation (2): $Y = 4.097ln(x)\ 2.173$

For EC_{50} calculation, the value for Y in the equations above is the 50% value of the highest nitrite concentration. Each of these values were substituted for Y in the equations and the value gotten for X became our EC_{50} values [1-2].

2.6. Analytical methods

The three effluent polluted soil samples were merged to form a composite sample, which were analyzed for the presence of PAHs alongside the non-polluted soil sample. The analysis was done using a PerkinElmer Gas chromatography with Mass spectrometry [15]. The Metabolite concentrations measurement such as nitrite and nitrate were determined using a visible spectrophotometry (JOD-778.000, England) [14].

3. Results and discussion

The impact of the hair dressing salon effluent on the soil sample was visible as a colour change was observed in the polluted soil samples, which include: the topsoil, midsoil and subsoil compared to the unpolluted soil sample, which served as the control soil sample (Plate 1, 2, 3 and 4). This study revealed some of the degradation by-products of different PAHs in the test soil sample. Most of these compounds are in the degradation pathway of many of PAHs like Biphenyl [15-16]. The following PAHs were present in the Salon effluent composting soil. The concentration of the PAHs present in the soil were 51.03ng/g Biphenyl, 15.01ng/g Benzo[a]pyrene, 31.32ng/g Anthracene, 6.42ng/g Phenanthrene and 5.02ng/g Naphthalene. Coronene and Fluorene were below the limits of detection. Only 1.90ng/g of Biphenyl detected in the control garden soil (Table 1). These results collaborated with [1-2] who monitored the impact of chemical constituent in hair dressing salon effluent on soil and soil bacteria. The presence of PAHs if not adequately controlled can affect the soil fertility and soil fauna, which has been established by [16-1-2]. These PAHs elicit toxic effects on the soil and soil biological sentinels, A previous research on hair dressing effluent had earlier revealed that autotrophic transformation by nitrifying bacteria, which enhances soil fertility, was hindered in an ecosystem polluted with high concentration of PAHs as nitrification processes were altered [1-2-12-16].

PAHs have been shown to have acute effects on heterotrophic bacteria as bacterial counts were progressively reduced in the effluent polluted soil as compared to the unpolluted soil sample, which recorded a higher bacterial count (Fig. 3). These results are in line with [12-16-23] who observed similar trend when they assessed the impact of plastics and herbicides on soil heterotrophic bacteria respectively. There was no significant difference in the bacterial counts recorded from the topsoil, midsoil and subsoil, which suggests that the effluent elicited relatively same impact across the test soil samples. It was observed that the acidic component in the effluent inhibited bacterial growth as the effluent concentration increased [13-17-18]. It also was observed that the toxicity of PAHs in hair dressing salon effluent on the nitrifying bacteria depended on the contact time and effluent concentration [19]. It was observed that there was an increase in the percentage inhibition of both bacteria (Fig. 2 c, d) with increased effluent concentration and time. There was an increase in nitrite production and utilization at lower effluent concentration, which began to decrease as the effluent concentration increased with time (Fig. 2a, b).

There was a progressive increase in the EC_{50} values of Nitrosomonas and Nitrobacter (Fig. 1B) which is the concentration required to give 50% response. This suggests that both nitrifying bacteria were able to survive the effluent toxicity at low effluent concentration which is in line with a previous study on hair dressing salon effluent concentrations on soil bacteria [1]. A progressive decrease in the LC_{50} of both nitrifying bacteria was observed, which suggests that at higher concentrations of the hair dressing salon effluent, both bacteria were inhibited (Fig. 1A). There was a statistical difference between *Nitrosomonas* sp. and *Nitrobacter* sp. toxicity response, which suggests that Nitrobacter sp., could be the preferred bacteria for the monitoring and assessment of hair dressing salon effluent toxicity. There was a significant difference between the nitrite accumulation by Nitrosomonas and nitrite utilization by Nitrobacter at ($p<0.05$). Comparing the bacterial inhibition, there was also a significant difference in the inhibition of both bacterial by the effluent at ($p<0.05$). From the values obtained, Nitrobacter showed a higher tolerance to the hair dressing salon effluent making it a preferable nitrifying bacterium for monitoring hair dressing salon effluent toxicity on soil, which agrees with [22-23].

Plate 1: Top Soil Sample

Plate 2: Mid Soil Sample

Plate 3: Sub Soil Sample

Plate 4: Non-polluted Soil Sample

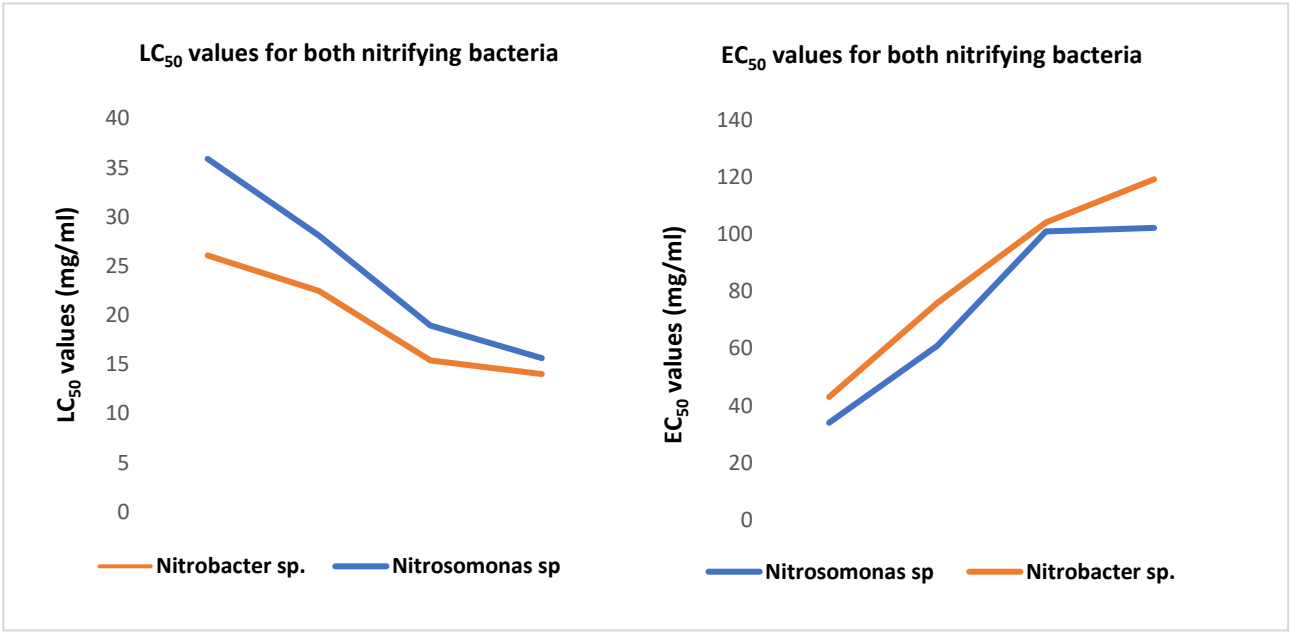

Figure 1. A: LC_{50} values for Nitrosomonas and Nitrobacter
B: EC_{50} values for Nitrosomonas and Nitrobacter

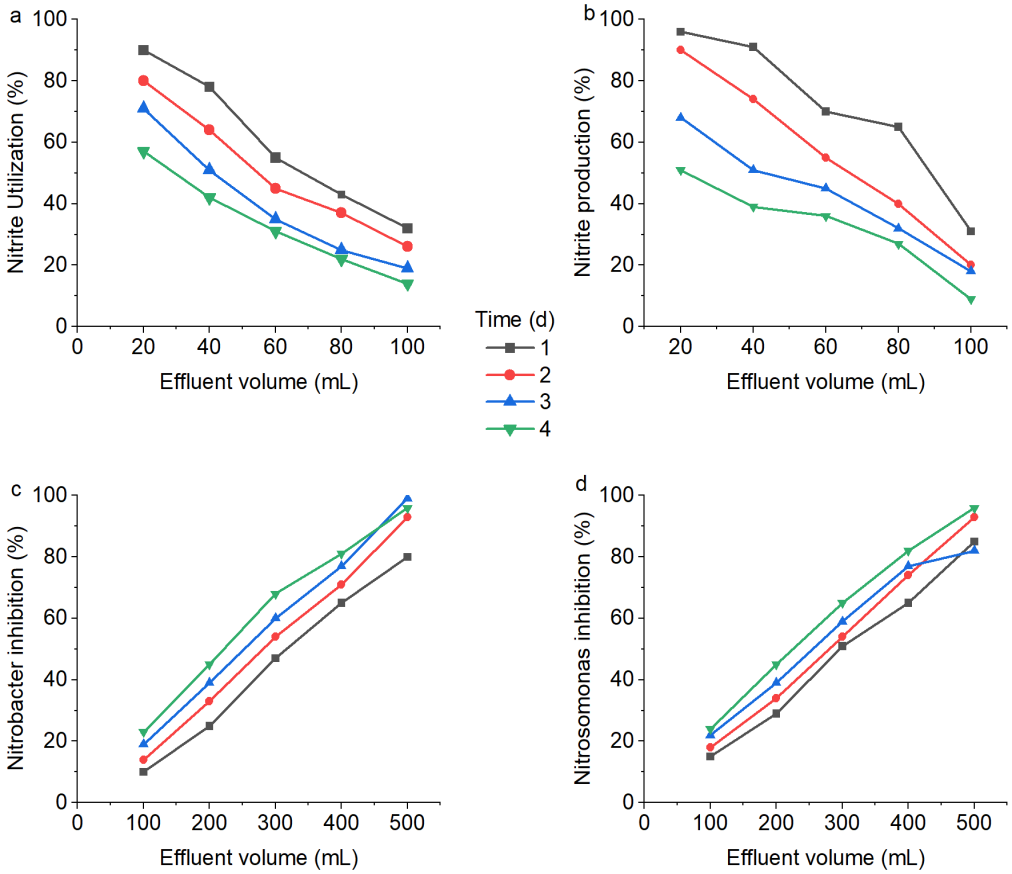

Figure 2. (a) Nitrite utilization by Nitrobacter. (b) Nitrite production by Nitrosomonas. (c) Percentage Nitrobacter inhibition in salon effluent (ml) from 1 to 4hrs. (d) Percentage Nitrosomonas inhibition in salon effluent (ml) from 1 to 4hrs.

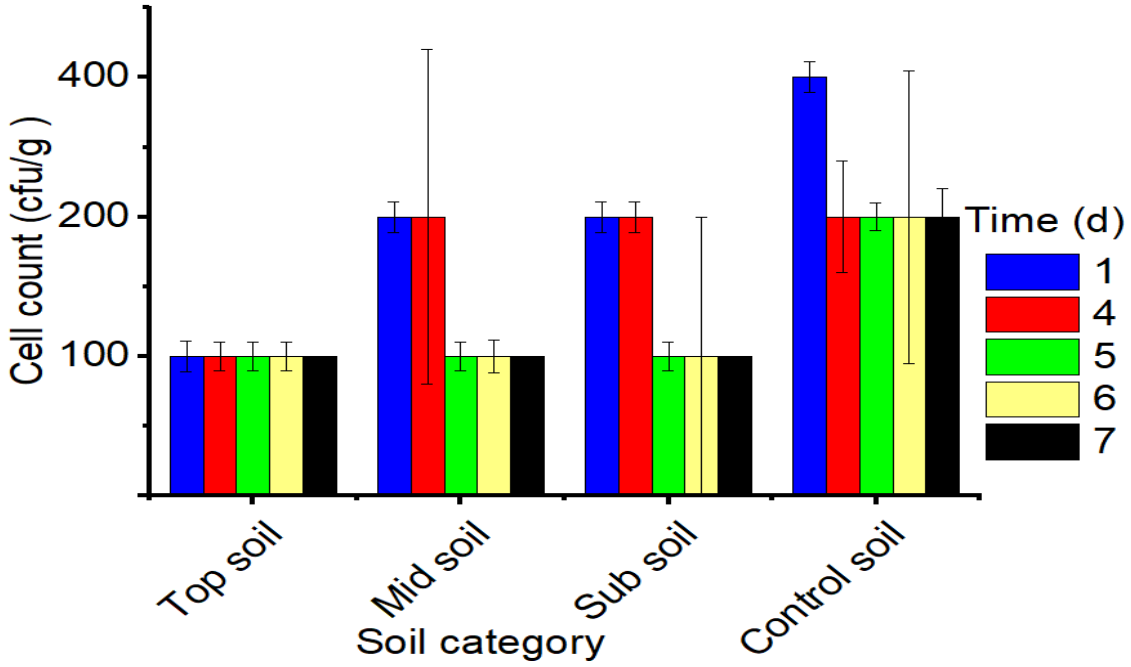

Figure 3. Heterotrphic bacterial counts for the four soil samples

Table 1. Individual PAHs detected in both soil samples

PAHs (ng/g)	Effluent composted soil	Garden soil sample
Biphenyl	51.03	1.90
Benzo[a]pyrene	15.01	NR
Anthracene	31.32	NR
Phenanthrene	6.42	NR
Naphthalene	5.02	NR
Fluorene	<LOD	NR
Coronene	<LOD	NR

Key: LOD: Limit of Detection,
NR: Not Recovered

Table 2. Metabolites concentrations

Time (hours)	Nitrite concentration (%)	Nitrate concentration (%)
1	51.21	46.07
2	47.50	32.07
3	31.22	25.10
4	28.65	18.11

4. Conclusion

The results from this study revealed the impact of polycyclic aromatic hydrocarbons from hair dressing salon effluent on soil nitrifying bacterial, which shows that hair dressing salon effluent seeps into the topsoil, midsoil, and subsoil eliciting toxic effects on the soil and soil biological sentinels. This research has identified a significant relationship between the chemical constituents of the salon effluent and soil nitrifying bacteria. Given that both bacteria are very important in the nitrification process, the results obtained from this study suggests that autotrophic transformation by nitrifying bacteria which enhances soil fertility may be hindered in an ecosystem continuously polluted with polycyclic aromatic hydrocarbons from hair dressing salon effluents as nitrification processes will be altered. This continuous disruption of nitrification could affect agricultural production and food supply.

Conflict of Interest

There is no conflict of interest whatsoever within the authors.

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