International Journal of Environment and Pollution Research
Vol.4, No.3, pp.28-39, July 2016
Published by European Centre for Research Training and Development UK (www.eajournals.org)

EFFECTS OF INDUSTRIAL EFFLUENT ON THE ENVIRONMENT USING *ALLIUM CEPA* AND *ZEA MAYS* AS BIOINDICATORS

Odutayo F.O.I, Oyetade O.B, Esan E.B and Adaramola F.B.

Department of Basic Sciences, Babcock University, Ilishan- Remo Ogun State, Nigeria.

ABSTRACT: *Indiscriminate handling and disposal of industrial effluents into the environment represents one of the major sources of environmental pollution which invariably affects the health of man, plants and animals. The toxic effect of effluent from a candy-producing industry was investigated in terms of root growth inhibition and overall phytotoxicity using bio-indicator plants; Allium cepa and Zea mays. The presence and concentrations of some metals of environmental concern as well as Polycyclic Aromatic Hydrocarbons in the effluent were also determined. The Allium cepa and Zea mays tests were carried out at concentrations of 1, 5,10,25,50 and 100% of the untreated effluent. There was statistical significant ($P<0.05$) inhibition of root growth in both plants, the shoot length measurement also showed significant difference ($P<0.05$) at the different concentrations of the effluent as compared with the control. Morphological abnormalities were observed in the roots of the bio-indicator plants, it is therefore recommended that industrial effluents be treated before being disposed into the environment.*

KEYWORDS: Industrial Effluent, Phytotoxicity, Allium Cepa, Zea Mays, Bio-Indicators, Environment.

INTRODUCTION

Environmental pollution is a major problem in many developed and developing countries around the world. Although pollution has been in existence for a long time, it was not until the development of industries and rapid organization around the 19th century that it really became a global problem (Gray, 2011). Environmental pollution in summary occurs when the environment cannot process and neutralize harmful by-products of human or industrial activities. Pollution reaches its most serious proportions in the densely settled urban-industrial centres of the more developed countries (Kromm, 1973). Pollution must be taken very seriously as it affects natural elements that are an absolute need for life to exist on earth. Many pollutants such as pesticides, oil, hydrocarbons, heavy metals as well as thermal and radioactive pollutants can get into the environment through direct or indirect release from industries, agriculture and households (Fathi et al., 2008). The excessive levels of these pollutants in the environment are causing a lot damage to human and animal health, plants and trees including tropical rainforest. (Tropical Rainforest Animals, 2008). The release of industrial effluents or wastewater into surrounding terrestrial or aquatic habitat has been implicated as one of the major sources of environmental pollution as industrial effluents are one of the by-products of human or industrial activities.

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ISSN 2056-7537(print), ISSN 2056-7545(online)

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LITERATURE REVIEW

Environmental pollution occurs when the environment becomes unable to process and neutralize harmful by-products of natural, human or industrial activities. It is a global problem which has been with man for decades and has become heightened majorly by the many industrialization and urbanization activities. These activities have led to the continuous generation of poorly handled and indiscriminately disposed harmful substances also known as pollutants. Many of these pollutants such as pesticides, oil, hydrocarbons, heavy metals as well as thermal and radioactive substances get into the environment through direct or indirect releases from industries, agricultural farmlands and domestic wastes (Fathi *et al*., 2008). The excessive levels of these pollutants in the environment are causing a lot of damages to human and animal health, plants and trees including tropical rainforest locations (Tropical Rainforest Animals, 2008). The indiscriminate handling and release of industrial effluents or waste water into surrounding terrestrial or aquatic habitat has been implicated as one of the major sources of environmental pollution. According to the United States Environmental Protection Agency (USEPA, 2006), effluent is defined as waste water treated or untreated that flows out of a treatment plant, sewer or industrial outfall. Industrial waste water varies both in flow and in pollution strength and can therefore be categorized as extremely complex mixtures containing inorganic and organic compounds (Nielsen and Rank, 1994). In Nigeria, over 80% of the industries release solid wastes, liquid effluents and gaseous emissions directly into the environment without any treatment (Federal Ministry of Water Resources [FEPA], 1991).

Industrial effluents carry various types of contaminants such as; metals, organic and inorganic matter, Polycyclic Aromatic Hydrocarbons (PAHs), microorganisms, etc. into the environment especially the aquatic systems (Ho *et al*., 2012).The complexity of industrial effluents as a result of its various contaminants makes it quite impossible to carry out a hazard assessment based on chemical analysis (El-Shahaby *et al*., 2003).Many studies (Fiskesjö, 1993; Babatunde and Bakare, 2006; Bakare *et al*., 2009; Oladele *et al*., 2011) have reviewed the possible genotoxic, cytotoxic, phytotoxic and overall growth reduction effects of industrial effluents on biological systems using biological tests which have given positive confirmations.

In this study, the phytotoxic and root growth inhibition effects of industrial effluent from a candy-producing industry was investigated using two biological tests; *Allium* test and *Zea mays* test. These two botanical systems have been chosen over animal models because they are equally sensitive, and are cheaper and easier to handle. They can be standardized and their response to environmental pollution can be easily evaluated. This study also sought to

ISSN 2056-7537(print), ISSN 2056-7545(online)

determine the metal and PAH contents of the industrial effluent which could predispose man and the environment to harmful or deleterious effects.

METHODOLOGY

Effluent collection and preservation

Raw (untreated) effluent was obtained from a Candy-production industry in Ibadan, Oyo State, Nigeria. The raw effluent was collected in a 1L pre-treated plastic container for metal analysis, 1L pre-treated glass container for PAHs analysis and a 5L plastic container for the purpose of the biological tests. The pH of the effluent was taken and later stored in the laboratory refrigerator until use.

Biological materials

The biological materials used for this study includes onions (*Allium cepa*; 2n=16)and maize grains (*Zea mays*; 2n=20). Medium sized onion bulbs were commercially obtained from Bodija market, Ibadan, Nigeria and were surface sun-dried for three weeks. The surface dried bulbs were later used to test for root growth inhibition. Over 100 yellow maize grains were also obtained from Bodija market, Ibadan, Nigeria and used to evaluate the phytotoxic potential of the industrial effluent in terms of seed germination and root and shoot length.

Analysis of Metal Content of Effluent Sample

200ml of the effluent sample was transferred into a 500ml beaker and 5ml of concentrated nitric acid was carefully added. The solution was heated on a water bath for a few hours in order to concentrate the solution to about 15ml. The resulting concentrate was allowed to cool and then filtered into a 25ml volumetric flask. The beaker was rinsed into the filtered paper with little quantity of distilled water and the solution was made up to mark. The filtrate was the transferred into a plastic vial for instrumental analysis. The heavy metals determined in the effluent; lead (Pb), cobalt (Co), zinc (Zn), copper (Cu), manganese (Mn), cadmium (Cd), magnesium (Mg), iron (Fe), nickel (Ni) and chromium (Cr) were analysed using Atomic Absorption Spectrophotometer (Buck Scientific Model 210 VGP) while flame photometer (Jenway FP 160 model) was used for the determination of Sodium (Na) and Potassium (K) and Phosphorus (P) was determined by the use of a spectrophotometer (Spectro SC Labmed model). A reagent blank was prepared and analysed following the same procedure with distilled water in the stead of sample

Analysis of PAHs Content of Effluent Sample

Analysis of PAH was carried out according to slightly modified EPA method.100mL of the effluent sample was transferred into a clean dry separatory funnel and mixed with 10mL dichloromethane. The mixture was shaken thoroughly and allowed to settle for proper phase separation. The organic layer was collected in a beaker and the process was repeated two more times with the same volume of dichloromethane. The combined organic phase was concentrated with a rotary evaporator at 40^0C down to about 1mL and the solvent was exchanged to hexane. Clean up of effluent extract was carried out through chromatography with a silica column. Saturated aliphatic hydrocarbons were eluted with 20mL of n-hexane and the aromatic hydrocarbons were eluted with 30ml of a mixture of hexane and dichloromethane (90: 10) (v/v). The volume of the eluted fraction was reduced to 1ml and the aromatic hydrocarbon fraction was injected into a gas liquid chromatograph (Agilent 7890A GC)

ISSN 2056-7537(print), ISSN 2056-7545(online)

equipped with a flame ionization detector (GC-FID) and an HP5 column was used in the analysis of the samples. The analysis was carried out in splitless mode with the oven temperature programmed at 100^0C initial temperature for 2minutes and then ramped to 280^0C at 10^0C/min. The temperature was held at 280^0C for 10 minutes.

Allium cepa test

The modified *Allium* test assay as given by Fiskesjö (1997) and Babatunde and Bakare (2006) were used for this study. First, the outer dried scales of the onion bulb and the brownish bottom plates (dried roots) were carefully scrapped off using a dissecting blade to reveal the ring of the primordial roots. Thereafter, the bulbs were planted in 100ml beakers containing the different concentrations (1%, 5%, 10%, 25%, 50% and 100%) of the effluent in a dark cupboard and at room temperature. Five onion bulbs were used for each treatment concentration. Tap water was used as the control of the experiment. The test concentrations were changed daily (i.e. every 24 hours) for continuous exposure. The onion bulbs were grown for 72 hours (i.e. three days) after which the root lengths of each of the onion bulbs treated with different concentrations of the effluents were harvested and measured for the analysis of root growth inhibition. The percentage of reduction in comparison to the control was obtained from the values recorded. The effects of the effluent on the morphology of the growing roots were examined as well.

Zea mays test

The modified guidelines for the testing of effluents on terrestrial plants according to the Organization for Economic Cooperation Development (OECD, 1984) were used for this experimentation. First, the viability of the seeds was determined by recasting the seeds in water to determine the floaters and the sinkers. The sinkers represent the viable seeds while the floaters represent seeds that might not grow. Surface sterilization of the final sinkers was then performed by immersing the seeds into 10% sodium hypochlorite or commercial bleach (i.e. 10ml bleach to 90ml water) for 5minutes to reduce contamination. Six maize seedlings was placed in a rectangular plastic containers lined with tissue paper containing 30ml of the effluent in different concentrations, while the rectangular plastic containers lined with tissue paper containing 30ml of distilled water served as the control. The experimentation of each concentration including the control was carried out in triplicates. The plastic containers were covered with a lid to prevent evaporation. The seeds were planted under room temperature for seven (7) days, after which the seed germination (%), shoot length (cm) and root length (cm) were measured. The shoot length measurement was taken from the base to the apical leaf of the plant using a transparent plastic ruler; while the root length was also measured by the same procedure after it was harvested and carefully washed with distilled water.

Statistical analysis

Data obtained from the *Zea mays* test and *Allium* root-growth inhibition test were expressed as percentage frequency and mean ± standard deviation using the one way ANOVA to test for significance between the control and the different concentrations at 95% confidence interval. Duncan's Multiple Range Tests Comparison at $p<0.05$ level of significance was used to compare the control and the different concentrations of different parameters measured.

RESULTS

The results of the mineral analysis of the effluent are presented in Table 1. These results showed the presence of eleven (11) out of the thirteen (13) metals assayed. Cadmium and Chromium were found to be absent in the effluent while others; Lead, Zinc, Nickel, Phosphorus, Calcium, Potassium, Sodium, Iron, Manganese, Magnesium and Copper were present at varied concentrations. The results of the metal contents of the effluent were compared with the stipulations of standard regulatory bodies; FEPA, WHO, US-EPA and especially NEQS standards for industrial effluents. The concentrations of Lead and Nickel in the effluent were found to be below the specified maximum permissible levels while the concentrations of Copper, Iron, Manganese and Zinc, were found to be considerably higher than those stipulated by the regulatory bodies especially by NEQS for industrial effluents. On the other hand, the concentrations of Potassium, Magnesium, Phosphorus, Calcium and Sodium were also found to be considerably high in the effluent.

The results of the PAHs contents of the effluent as shown in Table 2 revealed the presence of Naphthalene, Acenaphtylene, Acenapthene, Fluorene, Phenanthrene, Carbazole, Benzo[a]anthracene, Chrysene, Benzo[b]fluoranthene, Benzo[k]fluoranthene and Benzo[a]pyrene in the industrial effluent above the required limits while Anthracene, Fluoranthene, Pyrene, Indeno[1,2,3-cd]pyrene, Dibenz[a,h]anthracene and Benzo[ghi]perylene were absent. PAHs have been identified as toxic, carcinogenic and mutagenic and are considered pollutants of concern because of their adverse health impacts. The major polycyclic aromatic hydrocarbons present in the analysed effluent considered to be highly carcinogenic or genotoxic by the US-EPA, US Agency for Toxic Substances and Diseases Registry (ATSDR) and European Food Safety Authority (EFSA) include Benzo[a]pyrene, Benzo[b]fluoranthene, Chrysene, Benzo[k]fluoranthene and Benzo[a]anthracene (ATSDR, 1995; EFSA, 2008; Keith, 2014).

TABLE 1. METAL CONTENT OF THE INDUSTRIAL EFFLUENT

Metal	**Concentration in Effluent (mg/L)**	**FEPA (1991) limit (mg/L)** *for drinking water*	**USEPA limit(mg/L) (1998)** *for drinking water*	**NEQS, 2000 (mg/L)** *for Industrial Effluents*	**WHO, 1981 (mg/L)** *for drinking water*
Cadmium	None	0.01	0.01	0.1	0.05
(Cd)	None	0.05	0.10	1.0	0.05
Chromium	3.82 ± 0.19	0.01	0.05	1.0	-
(Cr)	118.36 ± 2.54	0.3	0.3	2.0	0.3
Copper (Cu)	2.32 ± 0.12	0.05	0.05	1.5	0.1
Iron (Fe)	0.0021 ± 0.7	0.05	0.01	1.0	-
Manganese	5.66 ± 0.18	5.0	5.0	5.0	5.0
(Mn)	0.36 ± 0.01	<1.0	0.05	0.5	0.05
Nickel (Ni)	64.53± 1.04	-	<20	-	12
Zinc (Zn)	90.73 ± 2.18	200	≤30	-	-
Lead (Pb)	3.28 ± 0.95	-	-	-	-
Potassium (K)	83.57 ± 4.15	200	75	-	-
Magnesium	274.03 ± 20.95	-	20		200
(Mg)					
Phosphorus					
(P)					

ISSN 2056-7537(print), ISSN 2056-7545(online)

Ph	4.80	-	6.0-8.5	6-10	6.5-9.2
Mercury	-	-	0.002	0.01	0.001
Arsenic	-		0.05	-	0.05

All values analysed are in mg/L. FEPA: Federal Environmental Protection Agency (1991). USEPA: United States Environmental Protection Agency (1998). WHO: World Health Organization (1981). NEQS: National Environmental Quality Standards (2000)

TABLE 2. PAHs CONTENT OF THE INDUSTRIAL EFLLUENT

PAHs	**Concentration in Effluent (mg/L)**	**Maximum limit (USEPA) mg/L**
Naphthalene	1.66096	-
Acenaphtylene	1.21478×10^{-1}	-
Acenapthene	1.81823×10^{-2}	-
Fluorene	5.40658×10^{-2}	-
Phenanthrene	1.01864×10^{-1}	-
Anthracene	Not found	-
Carbazole	1.66598×10^{-1}	-
Benzo[a]anthracene	2.71879×10^{-1}	0.0001
Chrysene	5.47824×10^{-2}	0.0002
Benzo[b]fluoranthene	3.61884×10^{-1}	0.0002
Benzo[k]fluoranthene	8.64775×10^{-2}	0.0002
Benzo[a]pyrene	5.54482×10^{-1}	0.0002
Indeno[1,2,3- cd]pyrene	Not found	0.0004
Dibenz[ah]anthracene	Not found	0.0003

***Allium* test**

The effects of the industrial effluent on *Allium cepa* was observed morphologically and also through root growth inhibition. Table 3 shows the results of the root growth inhibition effect of the industrial effluent on *Allium cepa*. The roots of onion bulb grown in the control (tap water) were whitish in colour, elongated and straight with no morphological deformities. Onion bulbs treated with 100% effluent showed black, rotten basal plates while short, scanty, spiral and yellowish root tips were observed at the other lower concentrations. At the various concentrations of the effluent, there was statistically significant ($p<0.05$) concentration-dependent inhibition of root growth with the highest mean growth at the control and least mean growth at 50% of the concentration.

TABLE 3. ROOT GROWTH INHIBITION ANALYSIS IN *ALLIUM CEPA*

Concentration (%)	Mean ± SE	Growth in % of control	Percent root growth inhibition
Control	4.70 ^{a}± 1.55	-	0
1	1.94^{b} ± 0.52	41.28	58.72
5	0.53^{c} ± 0.26	11.28	88.72
10	0.31^{c} ± 0.22	6.60	93.40
25	0.24^{c}± 0.08	5.11	94.89
50	0.0088^{c} ±0.0015	0.19	99.81
100	-	-	-

Mean of the same column are significantly different by Duncan's multiple range comparison test at P<0.05 (95% confidence interval)

***Zea mays* test**

The inhibitory effects of the industrial effluent on the growth of the shoot and root of maize as well as seed germination are represented in Tables 4. From the results of this study, the different concentrations of the industrial effluent had little or no effect on the germination of seeds as most of the concentrations had 100% growth but the rate of growth and length of roots differed according to effluent concentrations with the lowest shoot and root growth occurring at 100% concentration and the highest growth seen at the control. The inhibition of root and shoot growth was concentration dependent and statistically significant (P<0.05) at the tested concentrations in comparison with the control. Morphological deformities like stunted growth, curling up of leaves, discolouration and seed deterioration were also observed in the roots and leaves of the maize plants at the different concentrations.

TABLE 4. THE ROOT AND SHOOT LENGTHS (CM) OF MAIZE AT DIFFERENT CONCENTRATIONS.

Parameter	Concentration of Effluent (%)						
	Control	1	5	10	25	50	100
Root length (cm)	21.84^{a} ± 2.50	13.00^{c} ± 6.91	17.92^{b} ± 4.62	20.23^{ab} ± 2.34	16.92^{b} ± 3.01	6.97^{d} ± 2.88	0.41^{e} ± 0.13
Shoot length (cm)	16.38^{a} ± 2.74	13.44^{b}± 2.80	13.60^{b} ± 2.27	13.40^{b} ± 2.84	13.51^{b} ± 2.82	11.26^{b} ± 3.04	8.23^{c} ± 2.13

Mean of the same column are significantly different by Duncan's multiple range comparison test at P<0.05 (95% confidence interval)

DISCUSSION

From the results obtained from *Allium* and *Zea mays* tests, it was shown that the tested industrial effluent was phytotoxic and can affect growth and development through the inhibition of root in and shoot growth, and also induce some morphological abnormalities as shown in the *Allium* and *Zea mays* tests. The results of the *Allium* test shows complete root growth inhibition at the 100% concentration of the effluent indicating effluent toxicity and obvious morphological deformities like scanty, curled, short, spiral and coloured root tips at the other subsequent concentrations of the effluent. Results from previous reports (Bakare *et al*., 2009, 2013; Babatunde and Bakare, 2006) correlates with the observations recorded in this study. Further works done by Bakare, *et al*, (2009) has shown that there is a correlation between microscopic (genotoxicity) and macroscopic (morphological) parameters of *Allium cepa* as the effluent investigated in that study induced cytotoxicity, mutagenicity and genotoxicity. Reports by Babatunde and Bakare, (2006) and Fiskesjö (1997) also corroborates this study.

The fact that the effluent was obtained from a food producing industry could be responsible for the occurrence of some of these minerals at high concentrations. This is because food producing industries are known to make use of raw material such as table salt, sodium bicarbonate etc. which may be rich in some of these minerals. The results also showed that the pH of the effluent was 4.80, which is lower than the permissible pH level which range from 6.0 to 10 as stipulated by the standard regulatory bodies; FEPA, NEQS, WHO and USEPA. This implies that the effluent is very acidic and hence making its continuous discharge harmful to the soil and aquatic organisms. This is because, with this level of acidity, the effluents could greatly off-set the pH balances of both soil and water bodies, thereby making the soil unsuitable for planting and the waters unsafe for drinking or inhabitable for some aquatic organisms. Although the concentrations of few of the metals in the effluent fall below or within the maximum permissible levels stipulated by the WHO, US-EPA and NEQS, the continuous indiscriminate discharge of this effluent into lands and water bodies could spike up the concentrations of these minerals already existing in the lands and waters thereby resulting in serious environmental pollution. As a result, among other effects, surface and ground waters become polluted and soils become unsafe for agricultural purposes due to the phyto-absorptive ability of plants.

The presence of these metals discovered at high concentrations have been found to have negative effects on living organisms including the environment as they can induce cancer, damage the brain, heart, kidney and lung and increase degenerative diseases and ageing (Jordao *et al*., 2002). Also these metals when they permeate the soil can lead to contamination of the soil causing fluctuations in the availability of nutritive elements present in the soil. Some plants are known to accumulate metals like Fe, Zn, Mn, Cu, Pb, Cr and Cd from the soil leading to abnormal growth and visible morphological alterations like chlorosis, necrosis, stunted growth, shorter roots and narrower leaves (Ho *et al*., 2012). Metals especially the heavy metals cannot be decomposed and therefore store up in the environment leading to bioaccumulation and bio-magnification. High level of Chromium for example is also destructive as it is highly carcinogenic and it can generate different types of genetic effects in intact cells and in mammals' In-vivo (Khérici-Bousnoubra *et al*., 2009).

The structure of a PAH influences whether and how the individual compound is carcinogenic (Bostrom *et al*., 2002; Baird *et al.*, 2005). PAHs can be formed and found in the environment during biological processes or as products of the incomplete combustion of either natural combustion like forest fires or volcanic eruptions or man-made combustion of organic materials such as coal, oil and wood and also from industrial processes. Human exposure to PAHs can be due to inhalation, consumption of food and water and dermal contact in both occupational and non-occupational settings (Dong *et al*., 2012; Veltman *et al*., 2012). PAHs movement into the environment depends on how they are easily dissolved in water, and how easily they evaporate into the air. Plants can also absorb PAHs from soils through their roots and translocate them to other plant parts. Effects of PAHs on living systems depend on the route and length of exposure, the amount or concentration of PAHs being exposed to, and also

ISSN 2056-7537(print), ISSN 2056-7545(online)

the relative toxicity of the PAHs; American Conference of Governmental Industrial Hygienists (ACGIH, 2005). The short-term effects of PAHs on human include eye irritation, nausea, vomiting, diarrhoea and confusion (Unwin *et al.*, 2006) while the long term effects can include decreased immune function, cataracts, kidney and liver damage, breathing problems and lung function abnormalities.

Implication to Research and Practice

According to several authors (Wang et al., 2001; Mahmood *et al.*, 2005) germination, root elongation and shoot length are the most authoritative parameters that indicate changes in environmental quality. Data results obtained from the *Zea mays* test showed that the industrial effluent tested had an effect on the growth and development of maize. A similar study had been conducted by Gvozdenac *et al.* (2011) where germination, root and shoot length of selected plants were used as indicators of water quality and also by Orhue *et al*., (2005) where they studied the effect of brewery effluents on the growth of maize crop. The result of this study showed 100% seed germination at all concentrations except for 100% effluent and 1% effluent which had 83.30% and 88.90% respectively. This result showed a slight correlation with the study carried out by Fazal *et al*., (2007) in which the seed germination percentage was varied. The results obtained for shoot growth revealed that the effluent inhibits shoot growth showing variability in measurement with the lowest shoot length at 100% effluent and the highest shoot growth observed at the control. The root length also showed variation in its growth with the lowest measurement observed at 100% of the effluent and the highest length shown at the control. However, there was significant difference between the tested concentrations. Results from the study carried out by Gvozdenac *et al.* (2011) shows disparity in the shoot and root measurements at different degrees of water quality which correlates with the results gotten from this study corroborating the objective of this study. Morphological anomalies like chlorosis, curling, discolouration of the leaves were observed which may be due to the accumulation of metals on the leaves of maize.

CONCLUSION

According to this study, the industrial effluent from the candy producing industry induced phytotoxicity and root growth inhibition in *Zea mays* and *Allium cepa* respectively. The metals and PAHs determined to be present in the industrial effluent could be responsible for the toxicity of the effluent as perceived in the results of this study. The continuous indiscriminate disposal of these effluents to terrestrial and aquatic ecosystems may directly or indirectly increase the level of exposure of human beings to the toxic substances present in these effluents thereby predisposing them to harmful or deleterious effects. Therefore, adequate measures such as treatment of waste waters or effluents released after industrial production should be taken by industries so as to reduce the adverse environmental impacts of these effluents.

Future Research

The state of health and healthy living of human populace depend largely on the health status of the environment. This therefore places the responsibility on man to continuously and consistently monitor the environment. Urbanization and industrialization have negatively impacted the environment through the destruction of the ecosystem and pollution of the soil with industrial waste discharges. As part of our mandate, it is our plan to continuously assess the impact of human activities and proffer necessary solutions to seemingly unhealthy

ISSN 2056-7537(print), ISSN 2056-7545(online)

situations. Our future research work as usual will focus on improving the environment for better habitation and development.

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