Contents list available at Journal of e-Science Letters

Journal of e-Science Letters

journal homepage: http://scienceletters.researchfloor.org/

Heavy Metal and Microbial Contamination of Top Soil and Water around Wadata Abattoir, Makurdi Metropolis

Odo Joel Inya[2]*, Ebah Esther Eneyi[1], Joseph Yitmwa Gontur[1] and Ode Omenka Samuel[3]

[1]Department of Microbiology, University of Agriculture, Makurdi, Nigeria
[2]Department of Fisheries and Aquaculture University of Agriculture, Makurdi, Nigeria
[3]Department of Physics, Benue State University, Makurdi, Nigeria

ARTICLE INFO

Article History:
Received 21 July 2022
Revised 27 September 2022
Accepted 19 November 2022
Available Online 23 November 2022

Keywords:
Heavy metal,
Soil, Water,
Abattior,
Microbial Contamination,
Physicochemical Analysis

ABSTRACT

The analysis of the physicochemical parameters, heavy metal contamination and microbial contamination of top soil and water was carried out. The soil and water samples were collected from abattoir sites of Wadata, Makurdi metropolis; they were transported to the Laboratory, Microbiology Department Federal University of Agriculture Makurdi and were analyzed using standard methods. Physicochemical parameters such as pH, turbidity, temperature, conductivity and nitrate contents were all analyzed using standard methods. Heavy metals (lead and copper) contents of the samples were also analyzed using a spectrophotometer. The microbial analysis of the soil and water was also determined using a serial dilution method and pour plating. Identification of the bacterial isolates was done using the Bergy's manual of determinative bacteriology and characterization of the isolates was also done using standard methods. The result of the physicochemical parameters of the samples shows a pH range of 7.6-7.7 for the water samples and 8.5-9.0 for the soil samples. The nitrate content of the samples shows a range from 11-15 mg/L for both the soil and water samples and some of the values for the physicochemical parameters fall slightly above normal ranges while some were within normal ranges. Analysis of the heavy metals shows a trace amount of Lead and Copper in part per million (ppm) 0.64-0.68ppm for Lead and 0.16-0.19ppm for Copper. Microbiological analysis shows the presence of both Gram positive cocci (Staphylococcus sp) with a higher occurrence of 50% and Gram negative rods (E. coli and Klebsiella sp) both with 25% respectively and fungi species such as Mucor sp, Penicilium sp, Rhizopus sp Aspergillus sp and Paracoccidoides. The water at the slaughtering site has a higher colony count of 5.0×106CFU/ml while that at the butchering site has a count of 3.0×106 CFU/ml and the colony count of the soil at slaughtering site has a higher colony count of 4.4×106 CFU/g as compared to the soil at butchering site which has a least count of 4.12×106 CFU/g. The conclusion made from the result of this research was that the heavy metal contamination of the analyzed samples was minimal likewise the microbial contaminants. The recommendation made from the results of this research was that proper treatment of water from abattoir sites should be encouraged, and the disposal of the abattoir water should be away from water sources.

1. Introduction

Environmental pollution by heavy metals which adversely affect soil quality and pose a threat for human health requires a rapid and comprehensive solution. Numerous anthropogenic sources of pollutants can contaminate the soil and water environment, including inputs from waste waters flowing from mines and waste storage, runoff of pesticides from agricultural land or atmospheric deposition (Song *et al.*, 2010). Increasing industrialization has been accompanied throughout the world by the extraction and distribution of mineral substances from their natural deposits. The spread of heavy metals in the terrestrial environment is largely attributed to the disposal of waste products, mining activity and wastewater irrigation (Jillian *et al.*, 2015). Heavy metal pollution is becoming an increasingly serious environmental problem for soils. For example, the cadmium (Cd) content can reach

*Corresponding Author: Odo Joel Inya
E-mailAddress: odojoel@gmail.com
http://dx.doi.org/10.46890/SL.2022.v03i05.003

446 mg kg^{-1} in Japanese paddy soils (Herawati *et al.,* 2000), and 10,000 hectares of farmland are polluted and cannot be used for food production in Germany (Lewandowski *et al*., 2006). Rice tends to accumulate Cd and chromium (Cr) in its grains, the consumption of which can have toxic effects on living organisms. Therefore, the Cd content in rice is of great concern (Nagajyoti *et al.,* 2010; Soderland *et al.,* 2010). To date, investigations of the effects of Cd on rice plant ecosystems have mainly focused on the relationship between Cd pollution in paddy soils and Cd contents in rice. Microbial communities play important roles in soil because of the many functions they perform in nutrient cycling, plant symbiosis, and the detoxification of noxious chemicals used to control plant pests and plant growth (Nannipieri *et al*., 2003). High heavy metal levels in soil are a problem due to their toxicity to soil microorganisms and impairment of ecosystem functions. The short, medium, and long-term responses of microbial communities to heavy metal contamination have been investigated (Ranjard *et al.,* 2000; Sandaa *et al.,* 2001; Shi et al. 2002; Renella *et al.,* 2004). Heavy metals generally reduce the amount of soil microbial biomass (Brookes and McGrath 1984; Chander *et al*.,1995), leading to a decrease in functional diversity (Kandeler *et al*.,1996) and changes in microbial community structure (Frostegård *et al*., 1993; Gadd 1993; Elsgaard *et al.,* 2001; Sandaa *et al.,* 2001; Akmal *et al*., 2005). However, metal exposure may also lead to the development of metal-tolerant microbial populations (Ellis *et al.,* 2003). Due to their relationship to soil functionality, soil microbial populations and activity can serve as useful indicators of soil improvement and soil degradation (Ebah *et al*., 2022; Pankhurst *et al*., 1995; Dick *et al*., 1996; Shi *et al*., 2002; Zhou 2003; Verma and Singh 2005; Ivshina *et al*., 2014). Many studies have focused on the effects of heavy metals on root exudations, soil microbial activity, and soil enzyme activity and so on. However, little information is currently available about how heavy metals regulate the entire microbial community in the soil. Rice is the most important food crop in China. Recently, safe rice production became the key attention point due to the increasing heavy metal contamination problem. The aim of this study is to investigate the heavy metals and microbial contamination of top soil and surface water around Wadata abattoir in Makurdi metropolis.

Materials and methods

Study Area

This research was carried out in Makurdi, the capital of Benue state. Nigeria, West Africa. Its geographical coordinate are 7^0 14'0 North, 8^0 32'0" East and its original name Makurdi. The area is characterized by two seasons, the dry season (October to April) and rainy season (April to October) about 45% the total population are civil servant and business people. While about 25% are famers, 30% vocational workers and students.

Materials

The materials used in this research were; Soil samples, water samples, media [Nutrient Agar (NA), Petri dishes, wire loops, measuring cylinders, conical flask, spatula, etc.

Equipment

The following equipment were used during the course of the laboratory analysis of the samples, they include; Atomic Absorption Spectrophotometer (AAS), Microscope, Weighing balance, incubator, autoclave, refrigerator etc.

Reagents

The following reagents were used at the course of the laboratory analysis of the samples, these were; Gram reagents, urease reagents, Kovac's reagent, oil immersion, distilled water, citrate and 90% ethanol.

Sample Collection

Soil Sample Collection

The soil samples were collected from and around Wadata abattoir, Makurdi metropolis. They were placed in small lather bags and were sealed tightly; they were then transported to the Research Laboratory, Department of Microbiology Federal University of Agriculture Makurdi for analysis.

Water sample collection

Water samples were obtained in sample containers at the abattoir sites from and around Wadata, Makurdi metropolis, each sample was directly

collected into a factory-fresh 1.5 L plastic bottle, with cap securely tightened. After collection the bottles were immediately transported to the research Laboratory Federal University of Agriculture Makurdi for analysis.

Physicochemical Analysis of Soil and Water Samples

Physicochemical parameters such as temperature, pH, conductivity, turbidity, nitrate content were determined using standard instruments as described by APHA (2005)

Temperature was determined using a thermometer.

1. PH was measured using a pH meter.
2. Nitrate content was determined using nitrate meter
3. Electrical conductivity was measured using EC-meter
4. Turbidity was measured using sper turbidity meter

Heavy Metal Analysis in Soil

The soil were spread on a clean plastic sheet, placed on a flat surface and were air-dried in open air in the laboratory under room conditions for 24 h. Afterwards, the soil was sieved on a 2 mm sieve and 5 g. A sample was taken from the sieved soil and was added in a beaker. 10 ml of nitric/ perchloric acid, ratio 2:1 was added to the sample. This sample was digested at 105°C. Next, HCl and distilled water, ratio 1:1 was added to the digested sample and the mixture was transferred to the digester again for 30 min. The digestate was then removed from the digester and allowed to cool to room temperature. The cooled digestate was washed into a standard volumetric flask and was made up to the mark with distilled water. Determination of the heavy metals was done in an atomic absorption spectrophotometer (AAS model 210 VGP).

Sterilization and Disinfection of Materials

Work benches were properly disinfected with sodium hypochloride. All glass wares (petri plates, test tubes, conical flasks) were washed during the bench work with detergents and rinsed with clean water and will be sterilized at 121^0C.

Media Preparation

All media (Nutrient Agar (NA), Potato Dextrose Agar (PDA), and were prepared in accordance with the manufacturer's specification, homogenized and thereafter sterilized by autoclaving at 121^0C for 15 minutes. The media were then allowed to cool down to about 45^0C before use. Streptomycin (0.1%) was added to the Potato Dextrose Agar to prevent bacterial contamination of the media.

Bacteria and Fungi Isolation

Bacteria and fungi were isolated from the soil and water samples by serial dilution and pour plate technique on NA for bacteria and PDA for fungi. The isolates were macroscopically examined for morphology and colony characteristics such as the shape, surface, elevation, pigment, edge and opacity for bacteria and growth patterns, spore and mycelia coloration, distribution of spores for fungi. The microscopic examination was done by Gram staining and then viewed under oil immersion objective (x100 magnification) to see the Color, Shape, etc. for bacteria while the fungi were examined by lacto-phenol cotton blue staining and viewed under x10 magnification, then x40 magnifications to see the hyphae, spores, spore arrangement.

Identification and Characterization of Bacteria Isolates

The identification of bacteria was based on morphological characteristics and biochemical tests that were carried out on the isolates. Morphological characteristics such as colony appearance, consistency, colony surface and pigmentation were observed after 24 hours of growth.

Characterization was done according to the method proposed by Fawole and Oso (2004).

Gram Staining Technique

A thin smear of each of the 24hour old cultures was prepared on clean grease free slides, they were fixed by passing over flame gently. They were stained by the addition of 2 drops of crystal violet solution for 60seconds and were rinsed

with water. The smears were flooded again with Lugols' iodine for 30seconds and were rinsed with water, they were decolorized with 70% alcohol for 15seconds and were rinsed with distilled water. They were counter stained with two drops of safranin for 60seconds and finally were rinsed with water, they were then allowed to air dry. The smears were mounted on a microscope and were observed under oil immersion objective lens. Result was read according to color appearance. Gram negative cells appeared pink/red while Gram positive organisms appeared purple (Fawole and Oso, 2004).

Catalase test

A small quantity of the 24hour old culture was transferred into a drop of 3% hydrogen peroxide solution on a clean slide with the aid of a sterile inoculating loop. Result was interpreted based on the appearance of gas seen as white froth (Cheesbrough, 2006).

Coagulase Test

A loopful of the isolates was emulsified with normal saline solution on a microscopic slide. A drop of undiluted plasma was added to the suspension and stirred for five seconds. A coagulase-positive enzyme is indicated by the clumping of colonies together (Olutiola *et al.,* 2000)

Indole Test

Tryptone broth (5mL) was placed into different test tubes, after which a loopful of the bacterial isolates was inoculated into the test tubes, leaving one of the test tubes was not innoculated to serve as the control. The test tubes was then incubated at 37^0C for 48 hours, after incubation, 0.5 mL of Kovacs' reagent was added and shaken gently, it was allowed to stand for 20min to permit the reagent to rise. A red or red-violet color at the top surface of the tube indicates a positive result, while yellow coloration indicates a negative result (Cheesbrough, 2006).

Citrate Test

This test detects the ability of an organism to utilize citrate as a sole source of carbon and energy. About 2.4g citrate agar was dissolved in 100ml of distilled water. About 10ml of citrate medium was dispensed into each tube and covered, it was sterilized and allowed to cool in a slanted form. The tubes were inoculated by streaking the organisms once across the surface. A change from green to blue indicates utilization of citrate (Cheesbrough, 2006).

Urease Test

The surfaces of the urea agar slant were streaked with a portion of a well-isolated colony. The caps were leaved on loosely and the tubes were incubated at 35-37°C in ambient air for 48 hours to 7 days. The development of pink color was examined for as long as 7 days (Olutiola *et al.,* 2000).

Table 1: Physicochemical Parameters of Surface Water and Top Soil at Wadata Abattoir

Samples	Appearance	Temperature (°C)	Turbidity (NTU)	pH	Conductivity (µS/cm)	Nitrate (mg/l)
SS	-	23	12	9.0	300	15
WS	Turbid	24	13	7.6	400	11
SB	-	20	16	8.5	500	14
WB	Turbid	15	14	7.7	400	13

Key:

SS: Soil sample from slaughtering
SB: Water sample from slaughtering
WS: Soil sample from butchering
WB: Water sample from butchering

Table 2: Heavy Metal Analysis of Top Soil and Surface Water at Wadata Abattoir

Sample	Lead (ppm)	Copper (ppm)
SS	0.64	0.16
SB	0.65	0.17
WS	0.67	0.18
WB	0.68	0.19

Key:
SS: Soil sample from slaughtering
SB: Water sample from slaughtering
WS: Soil sample from butchering
WB: Water sample from butchering

Table 3: Colony Morphology and Appearances of Isolates from Surface Water and Top Soil at Wadata Abattoir

SAMPLES	CFU/ml	COLOR	ELEVATION	MARGIN	FORM	Texture
$SS10^{-2}$	5.2×10^{4}	Creamy	Raised	Entire	Circular	Smooth
$SS10^{-4}$	4.4×10^{6}	Creamy	Raised	Entire	Circular	Smooth
$WS10^{-2}$	7.24×10^{4}	Creamy	Raised	Entire	Circular	Smooth
$WS10^{-4}$	5.0×10^{6}	Creamy	Undulate	Entire	Circular	Smooth
$SB10^{-2}$	7.2×10^{4}	Creamy	Raised	Entire	Circular	Smooth
$SB10^{-4}$	4.12×10^{6}	Creamy	Raised	Entire	Circular	Smooth
$WB10^{-2}$	5.0×10^{4}	Creamy	Undulate	Entire	Circular	Smooth
$WB10^{-4}$	3.0×10^{6}	Creamy	Raised	Entire	Circular	Smooth

Key:
SS: Soil sample from slaughtering
SB: Water sample from slaughtering
WS: Soil sample from butchering
WB: Water sample from butchering

Table 4: Gram Stain and Biochemical Test of Isolates from Surface Water and Top Soil at Wadata Abattoir

Samples	Gram stain	Indole test	Catalase test	Citrate test	Urease test	Coagulase test	Suspected organisms
$SS10^{-2}$	-	-	+	-	-	+	*Klebsiella spp*
$SS10^{-4}$	+	-	+	-	-	+	*Staphylococcus spp*
$WS10^{-2}$	-	-	+	-	-	+	*Klebsiella spp*
$WS10^{-4}$	+	-	+	-	-	+	*Staphylococcus spp*
$SB10^{-2}$	-	+	-	+	-	-	*Escherichia coli*
$SB10^{-4}$	+	-	+	-	-	+	*Staphylococcus spp*
$WB10^{-2}$	+	-	+	-	-	+	*Staphylococcus spp*
$WB10^{-4}$	-	+	-	+	-	-	*Escherichia coli*

Key:
SS: Soil sample from slaughtering
SB: Water sample from slaughtering
WS: Soil sample from butchering
WB: Water sample from butchering

Table 5: Microscopic and Macroscopic Appearances of Fungi Isolates from Surface Water and Top Soil from Wadata Abattoir

Samples	Microscopic Appearance	Macroscopic Appearance	Suspected Fungi
WS	Presence of septated hyphae, colourless long unbranched sporangiospore with round radiate head of vesicle and brownish sclerotia	Dark brown powdery mycelium with dark spores on the surface and white to yellow colour on the reverse side.	*Aspergillus niger*
SB_a	Septate hyphae, colourless and brownish conidiophores with round radiate , lead biseriate phialides	Yellow-green colour with a creamy edge appearance on the surface, which appears golden to reddish brown on the reverse side.	*Aspergillus flavus*
SB_b	Septate and fruity mycelium and branched conidiophores with round radiate head vesicle and biseriate phialides	A rapid dark -green coloured growth with the edge surrounded by whitish margin on the surface and pale yellow on the reverse side.	*Penicillium spp*
SB_c	Presence of non-septate hyphae with a visible spore and short sporangiospore , mycelium branched	Creamy colonies that covers the entire medium. Irregular shape.	*Mucor spp*
SS_a	Presence of non septate branched mycelium with round shaped sporangia and filamentous hyphae.	Long hyphael growth cotton-candy like from the front white colonies which initially turns turns green to yellowish brown in lime the reverse is white to pale.	*Rhizopus spp*
SS_b	Presence of septate hyphae usually yeast and aleuriconidia are oval, unicellular truncate with a broad base and round apex.	The front colour is white cream or brown and the reverse colour is yellow brown brown.	Paracoccidoides

Key:
SS= Soil sample from slaughtering
SB= Water sample from slaughtering
WS= Soil sample from butchering
WB= Water sample from butchering

Table 6: Colony Frequencies and Percentages of Bacteria Isolates in the Samples

Isolates	Frequencies	Percentages (%)
Klebsiella sp	2	25
E. coli	2	25
Staphylococcus aureus	4	50
Total	**8**	100

Results

Physicochemical Parameters of Surface Water and Top Soil at Wadata Abattoir

The physicochemical analysis of surface water and top soil from Wadata Abattoir was done and recorded in Table 1. The analysis shows levels and ranges of parameters such as temperature, turbidity, conductivity, nitrate content and pH. The analysis of the soil shows that the soil sample SS had a temperature of 23 C; the turbidity was 12 NTU with pH 9.0. The conductivity and nitrate content were 300µS/Cm and 15mg/l respectively as shown in Table 1. The water sample WS has a turbid appearance and the temperature was 24 C,

turbidity was 13 NTU, pH was 7.6, conductivity and the nitrate content were 400 µS/Cm and 11mg/L respectively.

The colony morphologies of bacteria isolates in both the soil and water samples were obtained and recorded in Table 2, the Gram stain and biochemical test results were as recorded in Table 3. The morphologies and microscopic appearances of fungal isolates were also obtained and recorded in Table 4.

Discussion

It is very essential and important to test the water before it is used for drinking, domestic, agricultural or industrial purpose. Water must be tested with different physic-chemical parameters, likewise soil. This is because of the heavy metal contamination, microbial contamination and exceeding levels of physicochemical parameters (Sawane *et al.,* 2006). Selection of parameters for testing of water is solely depends upon for what purpose we are going to use that water and what extent we need its quality and purity. Water does contain different types of floating, dissolved, suspended and microbiological as well as bacteriological impurities (Saravanakumar *et al.,* 2011). Groundwater is the most important source of water supply for drinking, irrigation and industrial purposes. Increasing population and its necessities have lead to the deterioration of surface and sub surface water. The modern civilization and urbanization frequently discharging industrial effluent, domestic sewage and solid waste dump. The cause of ground water gets pollute and create health problems. Once the groundwater is contaminated, its quality cannot be restored by stopping the pollutants from the source it therefore becomes imperative to regularly monitor the quality of groundwater and to device ways and means to protect it (Aftab *et al.,* 2005)

In this study, the analysis of the physicochemical parameters, heavy metal (lead and copper) and microbial contamination of top soil and water was both carried out on samples from abattoir sides of Wadata, Makurdi metropolis. The analysis of the physicochemical parameters carried out on the soil samples at slaughtering side shows a slightly alkaline pH, the analysis of nitrates shows a level that was slightly above normal range and a high level of turbidity, conductivity was within a normal range. However the analysis shows that the soil samples have some levels of nitrates together with water samples. The conductivity values of both the soil and water at slaughtering and butchering sides falls within normal ranges, but the appearances were both turbid for the water samples. This result is in line with the standard by APHA (2005). The heavy metal analysis of the soil and water samples shows a minute quantity of lead and copper in part per million (ppm) the values for lead in the samples were 0.64ppm for sample SS, 0.65ppm for soil sample at butchering side , 0.67ppm for water sample WS and 0.68ppm for WB respectively. These values are not significant as they fall far below normal ranges. The heavy metal analysis of copper also shows a minute quantity of copper in part per million (ppm) in the samples. The values range from 0.16ppm -0.17ppm for the soil samples while the water samples has values ranging from 0.18ppm-0.19ppm for the water samples both at slaughtering and at butchering sides. This is in agreement with the work of Kostal *et al*. (2004). The microbial analysis shows the presence of *Klebsiella* sp, *Staphylococcus aureus* and *Escherichia coli*. The colony forming units of the coliforms ranges from 3.0×10^6 CFU/ml to 7.24×10^4 CFU/ml for the water samples while that of the soil samples range from 4.12×10^6 CFU/g to 7.2×10^4 CFU/g this finding is in agreement with that of Naseri *et al.* (2013).

Conclusion

The contamination of soil and water with heavy metals is a serious point of concern because of the effect on soil microorganisms and source of pollution. These metals contaminate the soil and water pose a threat to soil organism and aquatic lives. Apart from the danger on aquatic organisms, they are also detrimental to humans when ingested. Some of this metals are associated with serious ailments which brought about the need to investigate there soil and water contaminations. However the water and soil samples collected from abattoir sides in Wadata has minute and non significant levels of lead and copper. The conclusion made from the result of this research therefore is that the soil and water from abattoir sides of Wadata has minimal heavy metal contaminants and also restricted microbial species.

Recommendations

The recommendations made from the result of this study are;

1. The sitting of Abattoir sites away from water sources to avoid contaminants from animal remains.

2. Proper treatment of waste water before disposal to minimize heavy metal and microbial contamination.

3. Disposal of the water should be away from water sources to avoids mixing with contaminated water.

References

[1.] Aftab, Begum, S. Y, Noorjahan, C. M., Dawood, Sharif, S (2005), Physico-chemical and fungal analysis of a fertilizer factory effluent, Nature Environment and Pollution Technology, 4(4): 529-531.

[2.] APHA (2005) Standard methods for the examination of waste and waste water. 21st ed. American Public Health Association, Washington, D.C

[3.] Brookes P.C and McGrath S.P (1984) Effects of metal toxicity on the size of the microbial biomass. *J. Soil Sci.* 35: 341–346.

[4.] Ebah Esther Eneyi , Obochi Irene Ijakuwa1 , Odo Joel Inya (2022)Microbial and Physicochemical Assessment of Soil Contaminated with Cassava Waste Water in Makurdi Metropolis. International Journal of Health Systems and Medical Science. 1(4)

[5.] Ellis R.J, Morgan P, Weightman A.J, Fry J.C (2003). Cultivation-dependant and-independant approaches for determining bacterial diversity in heavy metal-contaminated soil. *Appl. Environ. Microbiol*. 69: 3223–3230.

[6.] Frostegård Å, Tunlid A, Bååth E (1993). Phospholipid fatty acid composition, biomass and activity of microbial communities from two soil types experimentally exposed to different heavy metals. *Appl. Environ. Microbiol*. 59: 3605–3617.

[7.] Herawati N, Suzuki S, Hayashi K, Rivai I.F Koyama H (2000). Cadmium,copper, and zinc levels in rice and soil of Japan, Indonesia, and China by soil type. Bull. *Environ. Contain. Toxicol*. 64: 33–39.

[8.] Jillian E.G, Robert S.B, Nishanta R (2015). Transfer of heavy metals through terrestrial food webs: areview. Environ. Monit. Assess. 187, 201.

[9.] Kandeler E, Kampichler C, Horak O (1996). Influence of heavy metals on the functional diversity of soil microbial communities. *Biol. Fertil. Soils.* 23, 299–306.

[10.] Kostal, J., Yang, R., W.u, C. H., Mulchandani, A., and Chen, W. (2004). Enhanced arsenic accumulation in engineered bacterial cells expressing ArsR. *Appl. Environ. Microbiol.* 70: 4582–4587.

[11.] Lewandowski I, Schmidt U., Londo M., Faaij A (2006). The economic value of the phytoremediation function-Assessed by the example of cadmium remediation by willow (Salix ssp). *Agr. Syst*. 89: 68–89.

[12.] Nagajyoti, P. C., Lee, K. D., and Sreekanth, T. V. M. (2010). Heavy metals, occurrence and toxicity for plants: a review. *Environ. Chem. Lett.* 8: 199–216.

[13.] Nannipieri P., Ascher J., Ceccherini M.T, Landi L., Pietramellara G., Renella G. (2003). Microbial diversity and soil functions. *Eur. J. Soil. Sci.* 54: 655–670.

[14.] Naseri, R.; Azadi, S.; Rahimi, M.J.; Maleki, A. and Mirzaei, A. (2013). Effect of inoculation with Azotobacter chroococcum and Pseudomonas putida on yield and some of the important agronomic traits in barley (Hordeum vulgare L.). *International Journal of Agronomy and Plant Production*, 4(7):1602-1610.

[15.] Pankhurst C.E., Hawke B.G., McDonald H.J., Kirkby C.A., Buckerfield J.C., Michelsen P., O'Brien K.A., Gupta VVSR, Doube B.M (1995). Evaluation of soil biological properties as potential bioindicators of soil health. *Aust. J. Exp. Agron*. 35, 1015–1028.

[16.] Ranjard L, Nazaret S, Gourbiere F, Thioulouse J, Linet P, Richaume A (2000). A soil microscale study to reveal the heterogeneity of Hg (II) impact on indigenous bacteria by quantification of adapted phenotypes and analysis of community DNA fingerprints. *FEMS Microbiol. Ecol*. 31, 107–115.

[17.] Saravanakumar, K. and R. Ranjith, Kumar, (2011), Analysis of water quality parameters of groundwater near Ambattur industrial area, Tamil Nadu*, India, Indian Journal of Science and Technology,* 4(5): 1732-1736.

[18.] Sawane, A. P., Puranik, P. G., Bhate, A. M., (2006). Impact of industrial pollution on river Irai, district Chandrapur, with reference to fluctuation in CO2 and pH, *Journal of Aquatic Biology,* 21(1): 105-110.

[19.] Song Y., Ji J., Mao C., Yang Z., Yuan X., Ayoko G.A., Fros R.L. (2010). Heavy metal contamination in suspended soils of Changjiang River – environmental implications. Geoderma, 159: 286–295.