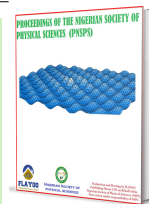


Published by Nigerian Society of Physical Sciences. Hosted by FLAYOO Publishing House LTD

Proceedings of the Nigerian Society of Physical Sciences

Journal Homepage: <https://flayooophl.com/journals/index.php/pnspsc>

Determination of the sources of heavy metals pollution using neem tree (*azadirachta indica*) stem barks in Maiduguri, Borno State, Nigeria

Abdullateef **Baba***, Abdullahi Idi **Mohammed**, Lawal Bukar **Inuwa**, Zaynab Mohammed **Chellube**

Department of Pure and Applied Chemistry, Faculty of Physical Sciences, University of Maiduguri, PMB 1069, Maiduguri, Borno State, Nigeria

ABSTRACT

Determining the sources of heavy metals pollution in the environment is very important in order to provide a solution to the problem. Plants are important indicators of heavy metals in Environmental Pollution. Neem trees is used for shade lining the streets or in most people's back yards in Maiduguri. This study was aimed at determining the concentration ($\mu\text{g/g}$) of Mn, Ni, Co, Cr, Cd, Cu, Fe, Zn and Pb in barks of Neem tree at various distances away from the main roads of Maiduguri Metropolitan council, Borno State, Nigeria. Samples (stem barks) were collected monthly for three months from three different locations (Bama station, Bulumkutu and Post office areas designated as S1, S2 and S3 respectively) at distances of 50m and 100m each from the main roads, and 250m to serve as control. The samples were collected monthly from the designated and control points for a period of three months. The concentrations of heavy metals in the samples were determined using Perkin-Elmer Analyst 200 Atomic Absorption Spectroscopy (AAS). The results showed that the mean concentrations ($\mu\text{g/g}$) of the heavy metals in the various locations varied for Zn ($0.115 \pm 0.007 - 0.719 \pm 0.003$), Mn ($0.234 \pm 0.07 - 1.413 \pm 0.134$), Cu ($0.013 \pm 0.001 - 0.151 \pm 0.003$), Co ($0.010 \pm 0.004 - 0.043 \pm 0.013$), Fe ($1.412 \pm 0.028 - 7.681 \pm 0.123$) and Pb ($0.003 \pm 0.003 - 0.245 \pm 0.147$). Analysis of Variance confirmed significant differences ($p < 0.05$) among the levels of the heavy metals from the three locations. Generally, the concentrations of the heavy metals decrease with increase in distance from 50 m to 100 m and then decrease at 250 m (control) from the main roads at locations S1 and S2. Hence, the pollution in S1 (bama station area) and S2 (Bulumkutu area) are due to both vehicular traffic and other anthropogenic activities while the pollution in S3 is due vehicular traffic activities. Therefore, the Neem tree bark can be used to determine the sources of heavy metals pollution in Maiduguri.

Keywords: Pollution sources, Heavy metals, Stem bark, Neem.

DOI:10.61298/pnspsc.2024.1.119

© 2024 The Author(s). Production and Hosting by FLAYOO Publishing House LTD on Behalf of the Nigerian Society of Physical Sciences (NSPS). Peer review under the responsibility of NSPS. This is an open access article under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/). Further distribution of this work must maintain attribution to the author(s) and the published article's title, journal citation, and DOI.

1. INTRODUCTION

Plants play very crucial roles in pollution control. According to their physical and chemical properties, contaminants can be ei-

ther stable or labile. The movement of the stoma, or mouth of the leaf, and trichome adsorption are what allow plants to carry out the absorption process (spines or leaf hair). Heavy metals are the group of inorganic chemical pollutants and road traffic emissions that are most harmful to the biosphere. Unlike organic pollutants, which can be broken down through biological and

*Corresponding Author Tel. No.: +234-806-1309-753.
e-mail: babsilega@gmail.com (Abdullateef Baba)

chemical processes, heavy metals tend to accumulate in the environment. Vehicular emissions and industrial exhausts harm the ecosystem while also causing heavy metal contamination. Untamed plants growing beside roadsides may assist in reducing heavy metal pollution. Heavy metals that impact the morphological, physiological, and reproductive characteristics of plants progressively change the pH of the soil. Roadside vegetation's germination and seedling development are impacted by heavy metal pollution. Phytoremediation can be employed as an alternative solution for heavy metal remediation processes because of its advantages as a low-cost, high-efficient, environmentally acceptable and eco-friendly techniques based on the utilization of metal accumulating plants. Future research on the number of heavy metals in a range of tropical roadside plants is necessary to determine the exact source and transport processes [1].

Azadirachta indica, commonly known as neem, margosa, nimtree or Indian lilac [2] is a tree in the mahogany family Meliaceae. It is one of two species in the genus *Azadirachta*. It is native to the Indian subcontinent and to parts of Southeast Asia, but is naturalized and grown around the world in tropical and subtropical areas. Its fruits and seeds are the source of neem oil. *Nim* is a Hindustani noun derived from Sanskrit *nimba* [3]. Margosa is a fast-growing tree that can reach a height of 15–20 metres (49–66 ft), and rarely 35–40 m (115–131 ft). It is evergreen, shedding many of its leaves during the dry winter months. The branches are wide and spreading. The fairly dense crown is roundish and may reach a diameter of 20–25 m (66–82 ft). The opposite, pinnate leaves are 20–40 cm (8–16 in) long, with 20 to 30 medium to dark green leaflets about 3–8 cm (1+1/4-3+1/4 in) long [4].

Assessment of Neem Tree (*Azadirachta Indica*) Leaves for Pollution Status of maiduguri Environment, Borno State, Nigeria, was carried out. It was found that the concentrations of some of the metals in the sampling points were lower than that of their corresponding controls. It was concluded that the Neem tree leaves do not only indicate pollution due to vehicular traffic activities but also other anthropogenic activities [5]. Ref. [6] used the bark of neem tree (*Azadirachta indica*) to monitor the levels of heavy metal pollution in Katsina Township. The result indicated a correlation between the heavy metal concentrations and distance of each site from the source of contamination with the exception of Cu, Pb and Zn concentrations. Amidst the heavy metal analyzed Fe has the highest level while Cu has the least. It was concluded that it may be due to the closeness of the sites to industrial activities or effluents emissions from domestic heating system, heavy traffic vehicles or emission from various roasting activities at these sites [6]. The efficiency for heavy metal remediation by *Panicum maximum* was investigated using pot plant experiments. The concentration of the heavy metals in *P. maximum* tissues decreased in the order root > stem > foliage. The phytoremediation of Pb²⁺, Cr³⁺ and Cd²⁺ contaminated soils with *P. maximum* seems to be promising under the conditions of the experiment. However, clear signs of phyto-toxicity appeared in the plants exposed to 120 ppm Pb²⁺ and Cd²⁺ at day twenty-three, suggesting that *P. maximum* may be a moderate metal accumulator [7]. Ref. [8] assessed the Phytoremediation Potential of *Chrysopogon Aciculatus* for some heavy metals (Cr, Co, Cd, Cu, Pb, Zn, Ni and Mn). It was observed that the grass plant

(*Chrysopogon aciculatus*) may have the ability of phytoextracting excess Mn from polluted soil. Ref. [9] investigated heavy metal levels in the bark of neem tree (*azadirachta indica*) some major roads in Ibadan, Nigeria. The concentration of the metal (mg/kg) ranged from: Cd (0.50 - 37.00), Cu (1.50 - 8.00), Fe (61.00 - 906.00), Pb (3.00 - 66.50) and Zn (6.40 - 810.50). The concentrations of the heavy metals in the control were relatively lower suggesting contamination of the studied environment. The concentrations of Cd, Cu and Zn (with exception in site E) were found below limit in all the sites. However, the concentration of Pb (with the exception in control site) and Fe (with exception in site A and D) were found to be higher than FAO/WHO maximum limits. Phytoremediation of some heavy metals in contaminated soil was carried out by Ref. [10]. General, heavy metals were more concentrated in roots than in shoots. The results implied that kenaf is having a higher efficiency for the removal of Cr, Co, and Cd, than flax. The potential of kenaf in removing the studied metal ions followed the descending order of Cr > Co > Mn > Cd where their removal percentage values reached 50.71, 38.27, 33.98, and 14.43%, respectively. For flax, the potential followed the descending order of Mn > Cr > Co > Cd, where their removal percentage values reached 54.36, 36.95, 28.72, and 11.37%, respectively. Phytoremediation efficiency of *Eleusine Indica* for Zinc, Copper, Lead, Nickel, Cadmium and Cobalt was investigated. The results of the investigation revealed that the elevated concentrations of the metals (Cu, Co, Pb, Zn, Cd and Ni) in roots and translocation to the aerial parts of the grasses suggest that the *Eleusine indica* is suitable for the Phytoremediation [11]. Levels of heavy metals in *Azadirachta indica* leaves for Monitoring Environmental Pollution in Guayaquil, Ecuador was studied. The results showed that the mean concentrations was in the order Zn > Cu > Mn > Ni > Cr in the sampling points. However, the concentrations of As, Pb and Cd were found below the limit of detection. It was concluded that these concentrations indicated the existing environmental contamination in the studied areas due to vehicular traffic and demonstrate the potential of the Neem leaves as an environmental bioindicator [12].

In Maiduguri, Nigeria, it is very common to see Neem trees used for shade lining the streets or in most people's back yards. In very dry areas the trees are planted in large tracts of land. Maiduguri is the capital and the largest city of Borno State in north-eastern Nigeria, on the continent of Africa. The city sits along the seasonal Ngadda River which disappears into the *Firki* swamps in the areas around Lake Chad [13]. Maiduguri was founded in 1907 as a military outpost by the British Empire during the colonial period. As of 2022, Maiduguri is estimated to have a population of approximately two million [14]. Maiduguri (Lat. 11°50'N, Long 13°10'E) is located in Borno State, Nigeria. It is underline by the sediments of Lake Chad basin. The hot season goes on for 2.4 months, from March 13 to May 26, with a typical everyday high temperature above 102 °F or 38.9 °C. The most blazing month of the year in Maiduguri is May, with a typical high of 103 °F or 39.4 °C and low of 79 °F or 26.1 °C. The cool season goes on for 2.1 months, from July 20 to September 23, with a typical everyday high temperature underneath 92 °F or 33.3 °C. The coldest month of the year in Maiduguri is January, with a typical low of 59 °F or 15 °C and high of 92 °F or 33.3 °C. The highest record temperature was 47 °C (116.6 °F) on 28 May

1983, while the lowest record temperature was 5 °C (41 °F) on 26 December 1979 [15]. The objective of the study is to determine the concentrations of heavy metals such as Cu, Co, Mn, Ni, Pb, As, Cd, Cr and Fe in barks of Neem tree samples at Post Office, Bulumkutu and Bama Station areas of Maiduguri Metropolis, in order to know the sources of the heavy pollution.

2. MATERIALS AND METHODS

2.1. SAMPLING AREA

The sampling locations were Bama station, Bulumkutu and Post office areas, Maiduguri, which were designated as S1, S2 and S3 respectively.

2.2. SAMPLE COLLECTION

Samples (barks) were collected using knife in locations S1, S2 and S3 within Maiduguri, Borno State, Nigeria. From each location, samples were collected at distances of 50 meters, 100 meters and 250 meters (control) away from main vehicular traffic roads. Sample collections were carried out according to the methods described by Ref. [16]; Samples were put into a pre-cleaned polyethylene bags and transported to the laboratory. The samples were collected monthly from the designated and control points for a period of three months. The samples were collected away from main roads at the distances 50 m, 100 m and 250 m in order to know the sources (vehicular traffic and/or anthropogenic activities) of the heavy metals pollution in the study area.

2.3. SAMPLE PREPARATION

Each of the Samples was dried separately in an oven at 105°C for 72 hours until they became brittle and crisp [17]. A portion (1g) of dried, disaggregated and sieved samples were placed separately in 50ml beakers and were digested with 10ml of HNO₃–HClO₄–HF (in the ratio of 9:4:1) to near dryness at 80 to 90°C on hot plate. The digested samples were filtered separately into a 50ml volumetric flask using Whatman No. 42 filter paper and made up to 100 mL mark with deionised water [16].

2.4. SAMPLE ANALYSIS

The digested samples were used to determine the concentration of Cu, Co, Mn, Ni, Zn, Cd, Cr, Fe and Pb using Atomic Absorption Spectrophotometer (Analyst 200 Pelkin Elmer).

2.5. DATA ANALYSIS

Data obtained was statistically analyzed using SPSS 16.0. Analysis of variance (ANOVA) with Turkey post-hoc test was used to determine the level of significance of variations between the samples. Results were considered statistically significant (P<0.05).

3. RESULTS AND DISCUSSION

Table 1 showed the heavy metal concentrations ($\mu\text{g/g}$) in the Samples at vary distances from the main roads in Bama Station area; table 2 showed heavy metal concentrations ($\mu\text{g/g}$) in the Samples at vary distances from the main roads in Bulumkutu area and table 3 shown heavy metal concentrations ($\mu\text{g/g}$) in the Samples at vary distances from the main roads in Post office area in Maiduguri metropolis. The samples contained variable levels of the heavy metals. Iron (Fe) has the highest concentration among the heavy metals studied.

The order of concentration of manganese from 50 m, 100 m and 250 m (control) from the three sampling points were S1>S2>S3, S1>S2>S3 and S3>S2>S1 respectively. The order of concentration of nickel from 50 m, 100 m and 250 m (control) from the three sampling points were S1>S2, S2>S3 and S1>S2 respectively. The order of concentration of cobalt from 50 m, 100 m and 250 m (control) from the three sampling points were S1>S2>S3, S3>S2>S1 and S1>S2>S3 respectively. The order of concentration of chromium from 50 m, 100 m and 250 m (control) from the three sampling points were S1>S3, S2>S3 and S1>S2 respectively. The order of concentration of cadmium from 50 m, 100 m and 250 m (control) from the three sampling points were S2>S1>S3, S3>S2 and S2>S1>S3 respectively. The order of concentration of copper from 50 m, 100 m and 250 m (control) from the three sampling points were S2>S1>S3, S3>S2>S1 and S1>S2>S3 respectively. The order of concentration of iron from 50 m, 100 m and 250 m (control) from the three sampling points were S2>S3>S1, S3>S2>S1 and S1>S2>S3 respectively. The order of concentration of zinc from 50 m, 100 m and 250 m (control) from the three sampling points were S1>S3>S2, S2>S3>S1 and S1>S3>S1 respectively. The order of concentration of lead from 50 m, 100 m and 250 m (control) from the three sampling points were S2>S3>S1, S1>S3>S2 and S1>S2>S3 respectively. Analysis of Variance (ANOVA) confirmed significant differences (p<0.05) between the levels of heavy metals within the stems from the different distances within the same location with the exception of cobalt, cadmium and lead in Bulumkutu. These might be due to the variations in the geographical status and traffic densities from different distances of the locations. Ref. [6] used Bark of Neem Tree (*Azadirachta indica*) as Bio-indicator for Monitoring Environmental Pollution in Katsina Township, Nigeria. He concluded that the result indicated a correlation between the heavy metal concentrations and distance of each site from the source of contamination with the exception of Cu, Pb and Zn concentrations. Amidst the heavy metal analyzed Fe has the highest level while Cu has the least. These are partly in line with the present study.

Generally, the concentrations of the heavy metals in the present study decrease with increase in distance from 50 m to 100 m and then decrease at 250 m (control) from the main roads at locations S1 and S2. However, the concentrations of the heavy metals increase with increase in distance from 50 m to 100 m and then increase at 250 m (control) from the main roads at locations S1. Hence, the pollution in S1 (bama station area) and S2 (Bulumkutu area) is due to both vehicular traffic and other anthropogenic activities while the pollution in S3 (Post office area) is due vehicular traffic activities. Therefore, the Neem tree bark can be used to determine the sources of heavy metals pollution in Maiduguri.

4. CONCLUSION

The samples contained variable levels of the heavy metals in which Iron (Fe) has the highest concentration among the heavy metals studied. Analysis of Variance (ANOVA) confirmed significant differences (p<0.05) between the levels of heavy metals within the stems from the different distances within the same location with the exception of cobalt, cadmium and lead in Bulumkutu. The concentrations of the heavy metals increase with

Table 1. Heavy metal concentrations ($\mu\text{g/g}$) in the barks at vary distances from the main roads in Bama station area.

Distance	Mn	Ni	Co	Cr	Cd	Cu	Fe	Zn	Pb
50 m	1.413 ^a ± 0.134	0.065 ^a ± 0.007	0.038 ^{ab} ± 0.011	0.062 ^a ± 0.062	0.017 ^a ± 0.007	0.049 ^a ± 0.005	1.751 ^a ± 0.040	1.268 ^a ± 0.003	0.003 ^a ± 0.003
100 m	0.317 ^a ± 0.029	N.D	0.010 ^a ± 0.004	N.D	N.D	0.013 ^a ± 0.001	1.412 ^b ± 0.028	0.211 ^a ± 0.013	0.245 ^a ± 0.147
250 m (control)	1.242 ^a ± 0.059	0.083 ^a ± 0.007	0.043 ^a ± 0.013	0.134 ^a ± 0.001	0.019 ^a ± 0.003	0.077 ^c ± 0.002	3.507 ^c ± 0.005	0.153 ^c ± 0.104	1.153 ^c ± 0.104

The above values are means of replicate values ($n = 3$). Within column, means with different alphabets are statistically different ($p < 0.05$). N.D= Not Detected.

Table 2. Heavy metal concentrations ($\mu\text{g/g}$) in the barks at vary distances from the main roads in Bulumkutu area.

Distance	Mn	Ni	Co	Cr	Cd	Cu	Fe	Zn	P ^a
50 m	1.753 ^a ± 0.077	0.013 ^a ± 0.009	0.032 ^a ± 0.008	N.D	0.023 ^a ± 0.009	0.109 ^a ± 0.002	6.093 ^a ± 0.077	0.160 ^a ± 0.010	0.089 ^a ± 0.089
100 m	0.429 ^b ± 0.030	0.068 ^a ± 0.003	0.025 ^a ± 0.010	0.026 ^a ± 0.026	0.002 ^a ± 0.002	0.048 ^a ± 0.001	5.504 ^a ± 0.159	0.719 ^a ± 0.013	0.030 ^a ± 0.004
250 m (control)	1.020 ^a ± 0.010	0.051 ^a ± 0.006	0.025 ^a ± 0.017	0.097 ^a ± 0.093	0.010 ^a ± 0.008	0.070 ^c ± 0.002	3.095 ^c ± 0.067	0.115 ^c ± 0.007	0.080 ^a ± 0.052

The above values are means of replicate values ($n = 3$). Within column, means with different alphabets are statistically different ($p < 0.05$). N.D= Not Detected.

Table 3. Heavy metal concentrations ($\mu\text{g/g}$) in the barks at vary distances from the main roads in post office area.

Distance	Mn	Ni	Co	Cr	Cd	Cu	Fe	Zn	Pb
50 m	0.234 ^a ± 0.070	N.D	0.012 ^a ± 0.004	0.017 ^a ± 0.005	0.041 ^a ± 0.001	0.109 ^a ± 0.002	1.858 ^a ± 0.006	0.215 ^a ± 0.001	0.072 ^a ± 0.025
100 m	0.978 ^b ± 0.076	0.052 ± 0.006	0.042 ^b ± 0.007	0.040 ^b ± 0.012	0.012 ^a ± 0.004	0.051 ^b ± 0.003	7.681 ^b ± 0.123	0.530 ^b ± 0.001	0.211 ^b ± 0.022
250 m (control)	0.880 ^b ± 0.187	N.D	0.016 ^a ± 0.012	N.D	0.003 ^b ± 0.001	0.015 ^c ± 0.001	2.832 ^c ± 0.133	0.132 ^c ± 0.010	0.022 ^{ab} ± 0.022

The above values are means of replicate values ($n = 3$). Within column, means with different alphabets are statistically different ($p < 0.05$). N.D= Not Detected.

increase in distance from 50 m to 100 m and then increase at 250 m (control) from the main roads at locations S1. Hence, the pollution in S1 (bama station area) and S2 (Bulumkutu area) are due to both vehicular traffic and other anthropogenic activities while the pollution in S3 (Post office area) is due vehicular traffic activities. Therefore, the Neem tree bark can be used to determine the sources of heavy metals pollution in Maiduguri.

References

- D. K. Mahida, V. M. Makwana, M. S. Sankhla, A. Patel & P. Dodia, "Accumulation of heavy metals in roadside plants and their role in phytoremediation", in *Anthropogenic environmental hazards*, P. Pathak, R. R. Srivastava, S. Ilyas (Ed.), Springer, Cham., 2023. https://doi.org/10.1007/978-3-031-41013-0_6.
- United States Department of Agriculture (USDA), "Azadirachta indica", Agricultural research service. [Online]. Retrieved 9 June 2017. <https://www.scirp.org/reference/referencespapers?referenceid=2668677>.
- Compact Oxford English Dictionary, "Neem", Oxford University Press, 2013, pp. 679. <https://www.gbif.org/species/113654962>.
- T. Olanrewaju, "Neem Azadirachta indica", Plants for a future. [Online]. Retrieved 25 October 2023. <https://sunnewsonline.com/orno-faces-new-challenges/>.
- A. Baba, B. G. Kolo & I. Waziri, "Assessment of neem tree (azadirachta indica) leaves for pollution status of Maiduguri environment, Borno State, Nigeria", The International Journal of Engineering and Science (IJES) **3** (2014) 31. <https://www.theijes.com/papers/v3-i9/Version-2/E0392031035.pdf>.
- S. A. Fowotade, S. A. Abdullah, A. A. Umar, I. Saleh & S. O. Oladeji, "Bark of neem tree (azadirachta indica) as bio-indicator for monitoring environmental pollution in Katsina township, Nigeria", ChemSci J. **9** (2018) 188. [10.4172/2150-3494.1000188](https://doi.org/10.4172/2150-3494.1000188).
- O. S. Olatunji, B. J. Ximba, O. S. Fatoki & B. O. Opeolu, "Assessment of the phytoremediation potential of *Panicum maximum* (guinea grass) for selected heavy metal removal from contaminated soils", African Journal of Biotechnology **13** (2014) 1979. <http://www.academicjournals.org/AJB>.
- S. T. Garba, M. A. Idi & A. Baba, "Assessing the phytoremediation potential of the grass; chrysopogon aciculatus for the heavy metals: Cr, Co, Cd, Cu, Pb, Zn, Ni and Mn", Global Journal of Science Frontier Research: H Environment & Earth Science **16** (2016) 14. https://globaljournals.org/GJSFR_Volume16/3-Assessing-the-Phytoremediation.pdf.
- S. O. Bankole, D. O. Alao, O. S. Ariwoola, O. A. Osunlaja, S. F. Oladejo, & C. Eccepacem, "Investigation of heavy metal status in neem tree (azadirachta indica) barks existing along some selected major roads in Ibadan, Oyo State", International Journal of Agriculture, Environment and Bioresearch **3** (2018) 4. www.ijaeb.org.
- M. Sherine, R. K. Shehata, K. Badawy & Y. I. E. Aboulsoud, "Phytoremediation of some heavy metals in contaminated soil", Bulletin of the National Research Centre **43** (2019) 189. <https://doi.org/10.1186/s42269-019-0214-7>.
- M. Gudusu, A. Tukur & S. Adam "Phytoremediation potential of *eleusine indica* for Zinc, Copper, Lead, Nickel, Cadmium and Cobalt", Nigerian Research Journal of Chemical Sciences **7** (2019) 65. <https://www.unn.edu.ng/wp>.
- M. J. Morales-Estupiñan, R. Sandra, O. Katherine & P. William, *Analysis of heavy metals in azadirachta indica A. Juss leaves, as bioindicator for monitoring environmental pollution in Guayaquil, Ecuador*, Proceedings of the 6th World Congress on New Technologies (NewTech'20), Prague, Czech Republic, 2020. [10.11159/icepr20.145](https://doi.org/10.11159/icepr20.145).
- T. Olanrewaju, "Borno faces new challenges", The Sun Nigeria. [Online]. <https://en.wikipedia.org/wiki/Maiduguri>.
- V. Hiribarren, *A history of Borno: Trans-Saharan African empire to failing Nigerian State*, Hurst & Company, London, 2017, pp. 106. <https://en.wikipedia.org/wiki/Special:BookSources/9781849044745>.
- Wikipedia-Maiduguri. [Online]. <https://en.wikipedia.org/wiki/Maiduguri>.
- M. Radojevc, & N. V. Bashin, *Practical environmental analysis*, Royal Society of Chemistry and Thomas Graham House, Cambridge, 1999, pp. 180-430. <https://www.scirp.org/reference/referencespapers?referenceid=2668677>.
- APHA (American Public Health Association) Standard Methods for the Examination of Water and Wastewater (16th Ed.), New York, 1992, pp. 75 - 86. <https://law.resource.org/pub/us/cfr/ibr/002/apha.method.3111.1992.html>.