COMPARATIVE ANALYSIS OF HEAVY METALS IN FISH SAMPLES COLLECTED FROM RIVER NIGER IN ANAMBRA STATE**.**

Abstract

Concern over heavy metal pollution has been widespread. Finding the levels of heavy metals in various fish species, including schilbe, semilabeo, giraffe, and elopsmachanata, from four fishing locations in the Anambra River was the goal of this study. After the fish samples were digested, Inductive Coupled Plasma Optical Emission Spectroscopy (ICP OES) was used to examine them for Fe, Cd, Cr, Ni, Pb, Sb, and Zn. The semilabeo fish sample during the dry season had the highest Fe concentration (23.42±1.38 mg/kg), while the elopsmachanata sample had the lowest concentration (9.213±1.768 mg/kg). Most of the Ni, Cd, and Pb were not detectable.

The ANOVA analysis of the locations revealed that position D was the most polluted. The majority of these values were below FAO/WHO permitted limits, according to the statistics. The customer may be at risk for health problems from certain of the fish samples. Continuous pollution monitoring of the Anambra River is required.

**Keywords**: Heavy metals, Schilbe, semilabeo, Giraffe, Elopsmachanata, Inductive Coupled Plasma Optical Emission Spectroscopy.

**1. INTRODUCTION**

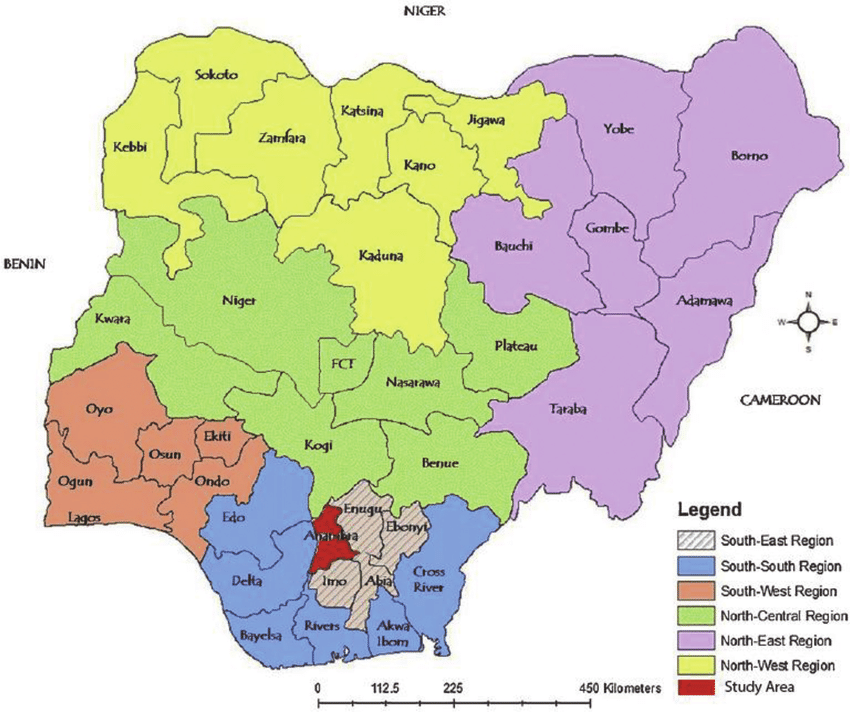
Fish, as aquatic creatures, serve as a primary source of protein for humans. Human activities consistently endanger their natural habitats, leading to environmental pollution. This pollution results in contamination and a decline in the quality of fish, posing risks to human health. [8]. Certain human activities stem from agricultural, domestic, and industrial practices [12]. Fish serves not only as a food source for people but also as a means of income. These pollutants endanger the food chain and diminish the revenue generated by this industry. The excessive buildup of these pollutants can result in fatalities or foodborne illnesses [4]. The River Niger, one of the largest rivers in Nigeria, along with other inland and waterways, is utilized for waste disposal. A significant portion of these wastes comprises heavy metals and various organic pollutants. [7]. Fish can accumulate heavy metals in various body parts, such as their tissues, and the degree of this accumulation is influenced by factors like their size, age, habitat, the chemical properties of the water, and their feeding habits. The health risks associated with consuming these contaminated fish include significant damage to vital organs like the kidneys, liver, brain, nervous system, and skin, potentially leading to death. As a result, this issue has become a worldwide concern over the years. This concern extends beyond just heavy metals to include toxic organic compounds, commonly referred to as pollutants. [11Among the 92 naturally occurring elements, roughly 30 metals and metalloids pose risks to human health, including Be, B, Li, Al, Ti, V, Cr, Mn, Co, Ni, Cu, As, Se, Sr, Mo, Pb, Ag, Cd, Sn, Sb, Te, Cs, Ba, W, and Pt. (Certain heavy metals, such as Arsenic, can be toxic even at low levels of exposure [15]. In recent years, there has been growing global concern regarding environmental pollution caused by these heavy metals. Many individuals have encountered this contamination through the widespread use of various industrial, agricultural, domestic, and technological applications [13].

Several ways in which heavy metals can enter our environments include the natural weathering of the earth's crust, mining activities, soil erosion, industrial discharge, urban runoff, wastewater from sewage, pest or disease control substances used on plants, and atmospheric pollution fallout, among others. The WHO/FAO has indicated that some of these heavy metals are vital nutrients necessary for various bodily functions. A deficiency or absence of these elements can lead to system malfunctions or even diseases. Some examples of these essential heavy elements include Cobalt (Co), Copper (Cu), Chromium (Cr), Iron (Fe), Magnesium (Mg), Manganese (Mn), Molybdenum (Mo), Nickel (Ni), Selenium (Se), and Zinc (Zn) [36]. Some of these elements can be spread by the food and/or drink we consume, but the majority can be spread via exposure. The acyclic chain order of heavy metal contamination includes industry, the atmosphere, soil, water, food, and humans. The rise in human exposure to heavy metals has a significant impact on the majority of developed nations. Aluminum (Al), antimony (Sb), arsenic (As), barium (Ba), beryllium (Be), bismuth (Bi), cadmium (Cd), gallium (Ga), germanium (Ge), gold (Au), indium (In), lead (Pb), Lithium (Li), Mercury (Hg), Nickel (Ni), Platinum (Pt), Silver (Ag), Strontium (Sr), Tellurium (Te), Thallium (Ti), Tin (Sn), Titanium (Ti), Vanadium (V) and Uranium (U)[14]. Among the heavy metals, there are five elements that are more dangerous than others. Laboratory studies demonstrate that oxidative stress and the generation of reactive oxygen species (ROS) are important factors in their toxicity and carcinogenicity. These metals include mercury [5], lead [1], chromium [6], arsenic [2], and cadmium [3]. Due to their high level of toxicity, these five heavy metals are important for public health. Even at low concentrations, these heavy metals have the potential to harm several organs. These five heavy metals are categorized as human carcinogens based on epidemiological and experimental research conducted by the International Agency for Research on Cancer (IARC) and the United States Environment Protection Agency (US.EPA). Every heavy metal has a unique set of characteristics and uses. They also have a different mechanism and degree of toxicity. Because of their high level of toxicity and review, these five heavy metals are the basis for this research's analysis to be conducted. This study will discuss a few heavy metals that are not commonly studied. These include lead (Pb), mercury (Hg), antimony (Ar), nickel (Ni), silver (Ag), iron (Fe), zinc (Zn), arsenic (As), cadmium (Cd), chromium (Cr), and cadmium (Cd).

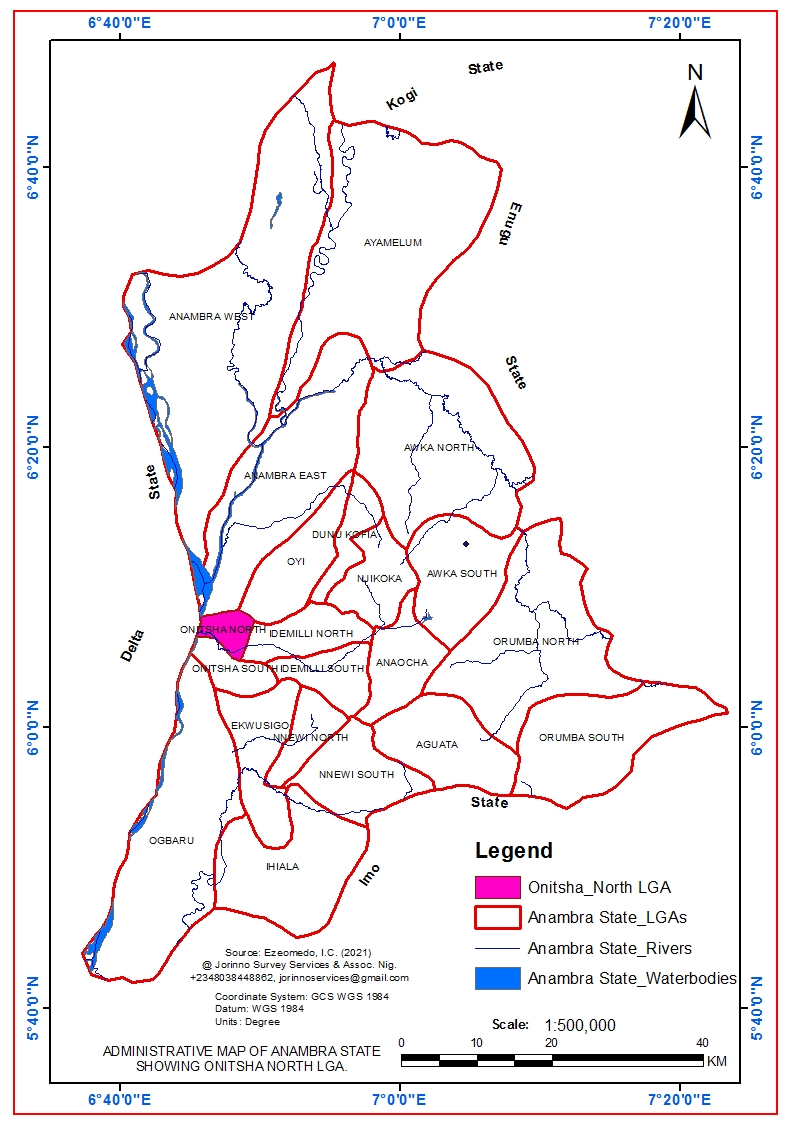
**2. MATERIALS AND METHODS:**

**Sample collection**:

At four distinct sites along the Anambra River in Niger with latitude and longitudes of 6.1581 and 6.7738 for site A, 6.1465 and 6.7704 for site B, 6.1740 and 6.7820 for site C and 6.0992 and 6.7579 for site D, three distinct fish species were gathered. The rainy and dry seasons were used to gather these various fish species.   
A fisherman assisted in the collection of these fish samples. The fish was collected, cleaned, and weighed. Prior to analysis, the samples were taken to the lab freezer for appropriate storage.



# Figure 1: Map of Nigeria showing Anambra State. (**Source: Ezeomedo, I.C. (2019)**



**Figure 2: Map of AnambraState Showing Onitsha North L.G.A. (Source: Ezeomedo, I.C. (2021)**

**Sample preparation:**

With the aid of a knife, the fish's intestine, flesh, skin, and gills were removed and digested. A digestion flask containing 20ml of aqua regia variation (acid combination, 65ml conc HNO3, 8ml perchloric acid, and 2ml conc H2SO4) was filled with 2g of each fish sample. A clear digest was obtained by heating the mixed sample in the flask to 650C on an electric hot plate. After being disconnected, the digest was allowed to cool in a desiccator. The digest was diluted with 100 milliliters of distilled water, filtered through Whatman filter paper, and then placed in a sample vial for the Perkin-Elmer Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) heavy metal analysis.

**Determination of metals:**

The Perkin Elmer ICP-OES was utilized. Standard operating procedure was the method employed. To ensure that the tissue was moving, the hood was examined. The computer was powered on with WINLAB 32 loaded and enough argon, the most prevalent component of plasma, for analysis. The software indicated that the ICP would be ready in 15 to 75 minutes after the chiller was turned on for five minutes. The sampler was automatically run with enough wash water, and the compressed air supply was reading 58 psi on the right gauge. After turning on the pump, the two sampling rods were submerged in the wash water. To create a bubble flow through the tube with a count of one to one thousand, the tensioning tube's rod was removed from the water. In order to see a smooth spray in the spray chamber, the knob was eventually adjusted and then repeated for the other tube.   
The 50 ppm Sc solution at the end of the lab bench was filled with the little sample rod. To make sure there were no water droplets on the sidewalls, the spray chamber was examined. After the tubing was arranged, the door was shut.

The analysis was ran using the manual process window. The results recorded on the log in the ICP information folder.

3. RESULTS AND DISCUSSION:

Table 1:

Mean concentrations of Heavy metals in different fish species in dry season.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Species of fish | Fe | Cd | Cr | Ni | Pb | Sb | Zn |
| *Schilbe* | 15.38±11.01 | 0.04±0.037 | 12.29±10.25 | 0.09±0.073 | 0.175±0.07 | 5.71±0.288 | 1.067±1.1 |
| *Semilabeo* | 23.42±1.38 | 0.045±0.026 | 16.13±15.25 | 0.07±0.21 | 0.32±0.209 | 5.12±0.887 | 4.185±6.03 |
| *Giraffe* | 18.16±11.98 | 0.035±0.013 | 10.61±12.03 | 0.05±0.04 | 0.163±0.08 | 5.445±0.458 | 0.898±0.52 |
| *Elops machanata* | 9.213±9.768 | 0.03±0.014 | 3.46±4.101 | 0.6±0.02 | 0.123±0.029 | 5.850±0.668 | 1.32±0.919 |

Figure 3: graph of Mean concentration of Heavy metal in different fish species in dry season

Table 2;

Mean concentration of Heavy metal in different fish species in raining season.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Species of fish | Fe | Cd | Cr | Ni | Pb | Sb | Zn |
| *Schilbe* | 13.986±11.018 | 0.035±0.038 | 16.737±6.539 | 0.440±0.441 | 0.135±0.035 | 4.823±1.370 | 0.983±0.354 |
| *Semilabeo* | 22.218±9.260 | 0.053±0.04 | 13.657±14.093 | 0.1±0.067 | 0.273±0.111 | 5.12±0.887 | 4.213±4.527 |
| *Giraffe* | 16.379±11.379 | 0.028±0.22 | 10.11±12.014 | 0.05±0.057 | 0.113±0.10 | 5.445±0.458 | 0.688±0.338 |
| *Elops machanata* | 9.595±8.598 | 0.023±0.005 | 3.493±4.518 | 0.048±0.027 | 0.11±0.035 | 5.850±0.668 | 1.239±0.737 |

**Figure 4: graph of** Mean concentration of Heavy metal in different fish species in raining season.

**Heavy metal concentration based on fish species**:

Table 1 shows the mean concentrations of all the fish species collected from River Niger in Anambra state in dry season. Fe has the highest concentration of 23.42mg/kg of heavy metal. Fe was generally highest in all the fish species, which means that it was the heavy metal that had accumulated in all the fish species analyzed. However, the hierarchy of the metal concentration detected in the fish samples was Fe˃Cr˃Sb˃Zn˃Pb˃Ni˃Cd with mean concentrations of 23.42,16.13,5.85,4.185, 0.6, and 0.045mg/kg respectively.

Nwude et al. [9] also reported that Fe (8.16-8.96 mg/kg), Zn (5.11-6.58 mg/kg), Cd (0.09-0.11 mg/kg), Cr (0.08-0.12 mg/kg), and Pb (0.35-1.30 mg/kg) were found in all the fish samples analyzed in the Ogun River, indicating that all the fish types contained heavy metal concentrations of which make them unsafe for consumption. Suynd et al. [4] made a similar observation regarding the metal contents of lake fish in an area near the disposal of industrial waste. The hierarchy of the metal concentrations detected in the fish samples was Fe˃Zn˃Pb˃Cr˃Cd. He added that all of the samples were the result of human activity on the mainland, such as the corrosion of water pipelines that go to the ocean and the discharge of home sewage that contains iron waste deposited by the industry. Additionally, Izuchukwu et al. [11] noted that fish samples from the Onitsha River in Niger had a high quantity of iron. It is consistent with these examined outcomes. As previously suggested by Suyud et al. [4], Izuchukwu put forth the argument that human activity is to be blamed.

Nwude et al. reported that the limit values of Pb and Cd are 0.02 and 0.05 mg/kg wet weight, respectively, while the European Emission Regulation [19] specifies maximum levels of 0.30 mg/kg for Pb and 0.05 mg/kg wet weight for Cd and FAO [16].[9] The levels in this study are typically above these thresholds, suggesting that eating fish from the area under examination at the time of the study could be harmful to the consumers' health. It also implies that the Niger River in Anambra State needs to be continuously monitored for pollution. At specific concentrations, several heavy metals are necessary for human growth. Fe and Zn are necessary elements. The immunological system, neurotransmission, and nucleic acid synthesis are all impacted by zinc [39]. Higher doses, however, have the potential to be harmful to fish [23] and to alter the carcinogenic reactions [18]. Acute zinc poisoning in humans can cause fever, diarrhea, vomiting, and nausea [35]. Fe is a common component of many businesses' effluents, which are frequently dumped into rivers. The oxidation state of iron may affect its toxicity to aquatic life. Fish are said to be more harmful to Fe2+ than Fe3+, for instance [28]. The human body needs Cr as a key element for the metabolism of carbohydrates [26]. On the other hand, Cr (Cr6+ ) may penetrate cell membranes and has a strong oxidative potential [27].

and is regarded as hazardous as a result. The suppression of some enzyme systems involved in the creation of cellular energy is one of the toxicological mechanisms of action of toxic heavy metals, which can also be mutagenic and carcinogenic [20]. Other harmful elements are Pb and Cd. One of the priority dangerous substances is lead. It damages the brain, causes cardiovascular illness, affects the central and peripheral neurological systems, and has negative effects on the fetus's developmental phases [29, 33]. With mean values of 23.42 mg/kg, semilabeo has the highest concentration of any heavy metal examined during the dry season. All of the heavy metal concentrations that were examined in the samples were rated in the following order: semilabeo > giraffe > schilbe > elopsmachanata, with respective concentrations of 23.42, 18.16, 15.38, and 9.21 mg/kg. According to data from the reviewed literature, the concentration of metals in fish filets varies greatly depending on the species and region [21], as well as the degree of water body contamination. The extent to which a specific species scavenges materials from the sediment and water determines how much heavy metal buildup occurs [22, 34]. Variations across species and the extent of toxicant accumulation may also be influenced by ecological requirements, eating patterns, and food metabolism [31]. The species that remain at the surface for a longer period of time [32] For instance, species, sample locations, pH, alkalinity, hardness, and the extent of its absorption into the sediments or the water's organic matter content are the main determinants of lead concentration and bioavailability in aquatic environments [25, 24, 30].

It was found that the same pattern manifested itself as examined in the dry season when looking at Table 2, which shows the same mean concentration of heavy metals from various fish species during the rainy season. The level of pollutants varies between the two seasons. Heavy metal concentrations were found to be higher during the dry season than during the rainy season. The total mean concentrations during the rainy season were Fe (15.55 mg/kg), Cd (0.03 mg/kg), Cr 11.00 mg/kg), Ni (0.16 mg/kg), Pb (0.16 mg/kg), Sb (5.31 mg/kg), Zn (1.78 mg/kg), and Cd (0.04 mg/kg), Cr (10.62 mg/kg), Ni (0.2 mg/kg), Pb (0.20 mg/kg), Sb (5.53 mg/kg), and Zn (1.87 mg/kg).

Table 3: Mean concentrations of heavy metals based on different locations in Anambra River in dry season

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Locations | Fe | Cd | Cr | Ni | Pb | Sb | Zn |
| *A* | 23.048±11.383 | 0.025±0.012 | 14.348±14.784 | 0.060±0.038 | 0.17±0.061 | 5.303±0.323 | 1.285±0.524 |
| *B* | 5.75±4.816 | 0.03±0.008 | 6.888±10.107 | 0.05±0.023 | 0.268±0.255 | 5.390±0.915 | 4.02±6.158 |
| *C* | 10.735±7.077 | 0.033±0.009 | 4.318±3.402 | 0.080±0.050 | 0.150±0.035 | 5.265±0.466 | 0.71±0.228 |
| D | 26.612±11.846 | 0.063±0.021 | 16.925±10.431 | 0.08±0.038 | 0.193±0.063 | 6.173v0.210 | 1.465±0.634 |

Table 4: Mean concentration of heavy metals based on different locations in Anambra river in raining season

Figure 5: graph of Mean concentration of heavy metals based on different locations in Anambra river in raining season

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Locations | Fe | Cd | Cr | Ni | Pb | Sb | Zn |
| *A* | 18.953±8.474 | 0.02±0.014 | 13.697±13.373 | 0.233v0.336 | 0.17±0.071 | 4.448±0.804 | 1.490±0.797 |
| *B* | 19.218±21.608 | 0.018±0.005 | 6.165±8.571 | 0.243±0.393 | 0.188±0.167 | 4.988±1.281 | 1.078±0.269 |
| *C* | 9.573±6.960 | 0.03±0.008 | 8.02±8.841 | 0.06±0.037 | 0.06±0.037 | 4.495±0.821 | 3.205±5.201 |
| D | 26.52±7.986 | 0.07±0.039 | 16.115±10.843 | 0.103±0.081 | 0.138±0.038 | 5.313±0.966 | 1.35±0.715 |

Table 5: Mean concentrations of heavy metals based on different locations in Anambra river in dry season.

Figure 6: Graph of Mean concentration of heavy metals based on different locations in Anambra River in dry season.

Heavy metal concentration based on location:

Tables 3 and 4 provide the specific findings of the average heavy metal concentration per location. For places A through D in the Anambra River, the mean concentration during the dry season is noted. The mean Fe content across all locations is 16.536 mg/kg, with location D having the highest value at 26.612 mg/kg and location B having the lowest at 5.75 mg/kg. The clayey material that can make up the river bed could be the cause of the high iron content. According to [41], it might also be the result of human activity like the release of untreated sewage that contains iron. Additionally, it has been noted that Nigerian soils contain high levels of iron. [42] had conducted similar studies in Lagos Lagoon and discovered high iron concentrations. Pb and Cu were also found in the sediment, but no antimony or cadmium was found.

Location D had the highest concentration of Cd (0.06 mg/kg), while location A had the lowest value (0.025 mg/kg). Location D had the greatest value of Cr (16.925 mg/kg), while location D had the lowest value (4.317 mg/kg). Locations C and D had the highest concentrations of Ni (0.08 mg/kg), whereas site B has the lowest. Location C has the lowest concentration of lead (0.150 mg/kg), whereas location D has the highest concentration (0.193 mg/kg). Additionally, location D has the highest concentration of Sb (6.173 mg/kg), whereas location C has the lowest value (5.265 mg/kg). Location B had the highest concentration of zinc, whereas Location C had the lowest concentration, 0.71 mg/kg. In their analysis of the heavy metals in fish, water, and sediment in the Anambra River, Izuchukwu et al. (2017) also found all of the heavy metals found in this study. All of the heavy metals he found in the fish samples, with the exception of lead, had concentrations that were within acceptable bounds [11].

The issue of aquatic food contamination may be resolved by managing or keeping an eye on the possible sources of heavy metals in the aquatic ecosystem. Toxic metal exposure may be higher in poor nations than in industrialized ones; this could be due to improper waste management or disposal [17] or pervasive pollution [38]. The majority of these heavy metal concentrations were higher than what was advised for fish samples. Therefore, it is necessary to regularly and consistently assess the Anambra River's heavy metal concentration over time.

**Limitations of the studies:** while this study provides valuable insight into the comparation of heavy metals in the fish species, some limitations were acknowledge. Small sample size, limited sampling locations and short time exposure.

**Conclusion:**

Fe, Cd, Cr, Ni, Pb, Sb, and Zn levels and presence were assessed in fish samples and at various fishing locations. The FAO/WHO acceptable limits were exceeded by the majority of the results. Customers may be at risk for health problems from certain of the fish samples. Continuous pollution monitoring of the Anambra River is required. These pollutants may be caused by commercial and industrial operations that produce garbage, including heavy metals, which are easily dumped into the Anambra River.

**Data availability**: The data supporting the findings of this study are available from the corresponding author upon reasonable request.

**Declarations**

**Competing interests**: The authors declare no competing interest.

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