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Spatial variations of the concentration of heavy metals (Cd, Pb) in the *Crassostrea gigas oyster* from Lake Nokoué in southern Benin

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Abstract
Lake Nokoué is located between parallels 6°20' and 6°30' North and meridians 2°20' and 2°35' East. This ecosystem is subject to a strong anthropogenic influence due to direct discharges and polluting inputs of urban and industrial origin drained by rainwater and sea currents. This study consisted of collecting oyster samples from three sites in Lake Nokoué and determining the Cd and Pb levels. The pH, salinity, dissolved oxygen, conductivity and temperature of the water were was also measured at the three stations using a portable HANNA HI 9829 type device to assess the state of the quality of the environment of these organisms. Oyster samples were mineralized and extracted using citrate buffer and a chloroform + dithiver mixture in a 500 mL ampule and measured using a Photolab brand molecular absorption spectrophotometer 6600 UV-VIS. The results show that the waters of Lake Nokoué is influenced not only by marine currents via the Cotonou Channel, but also by continental inputs from the Ouémé River and the Sô River and that the physicochemical conditions are favorable to the development of aquatic organisms. The maximum values of Cd and Pb recorded are respectively 10.40 mg/kg DM (Djidjè site) and 9.39 mg/ kg DM (Agbato site) greatly exceeding the reference values of the French standard set by decree of May 21, 1999 and Regulation (EC) No. 1881/2006. The sanitary quality of the aquatic species of Lake Nokoué in general and that of the oysters in particular must be rigorously monitored because they are highly appreciated by the populations.

Keywords: Contamination, heavy metals, physicochemical quality, oysters, Lake Nokoué

Introduction
Water is a component of the environment that is very crucial for the survival of living beings on the planet. The volume of water on the surface of the earth is estimated at nearly 1.4 billion km^{-3} (UNESCO, 2006) [1] and at 0.27% the share in the form of water in lakes and rivers (Morel, 2007) [2]. However, heavy metals are constituents of the earth's crust (Senez- Mello *et al*., 2020) [3] and of anthropogenic origin transported by water and atmospheric currents (Farrell *et al*., 2018) [4] and which accumulate in sediments, the water column and in living aquatic organisms, while the survival of several aquatic organisms depends on variations in physico-chemical conditions such as temperature, salinity, turbidity and conductivity (Senez-Mello *et al*., 2020) [3]. Oysters generally live in mangroves (Senez- Mello *et al*., 2020) [3] which are brackish water ecosystems subject to variations in conditions due to continental and marine waters.

In Benin, Lake Nokoué is one of the bodies of water of great socio-economic interest. The populations use it for their agricultural, transport and livestock activities, but also and above all for fishing. They manage to obtain a panoply of fish species including shrimp, fish, crabs and oysters. The latter have not only food and economic benefits, but also ecological and scientific ones (Agadjihouede *et al*., 2017) [5]. Their systematic position is as follows: Kingdom: Animal; Phylum: Mollusc; Class: Bivalve; Order: Ostreoida; Family: Ostreidae; Subfamily: Crassostreinae; Genus: *Crassostrea*; Species: *Gigas*.

Unfortunately, several anthropogenic activities around this lake cause water pollution (Širi´c *et al*., 2022) [21]. Thus, Youssao *et al* (2011) [7] found in Lake Nokoué a lead content in the sediments of 130.77 mg/kg and which is almost one hundred (100) times higher than that obtained in the water. (1.45 mg/L).

According to the study, fish (*Sarotherodon melanotheron*, *Tilapia guineensis* and *Hemichromis fasciatus*) *have* lead concentrations above the limit value of 0.4 $mg.kg^{-1}$ fresh weight set by the WHO (1995) [8]. Mercury residues were also evaluated up to a level of by Youssao *et al*. (2022) [9].
It is in this logic that the present study focuses on lead and cadmium contamination in oysters from Lake Nokoué.

Material and Methods

Description of the study area

The study area is Lake Nokoué located between parallels 6° 20' and 6° 30' North (Figure 1) and meridians 2° 20' and 2° 35' east. It is 20 km long from east to west (Kétonou - Abomey- Calavi) and 11 km wide in its north-south direction (Ouedo-gbadji - Cotonou).

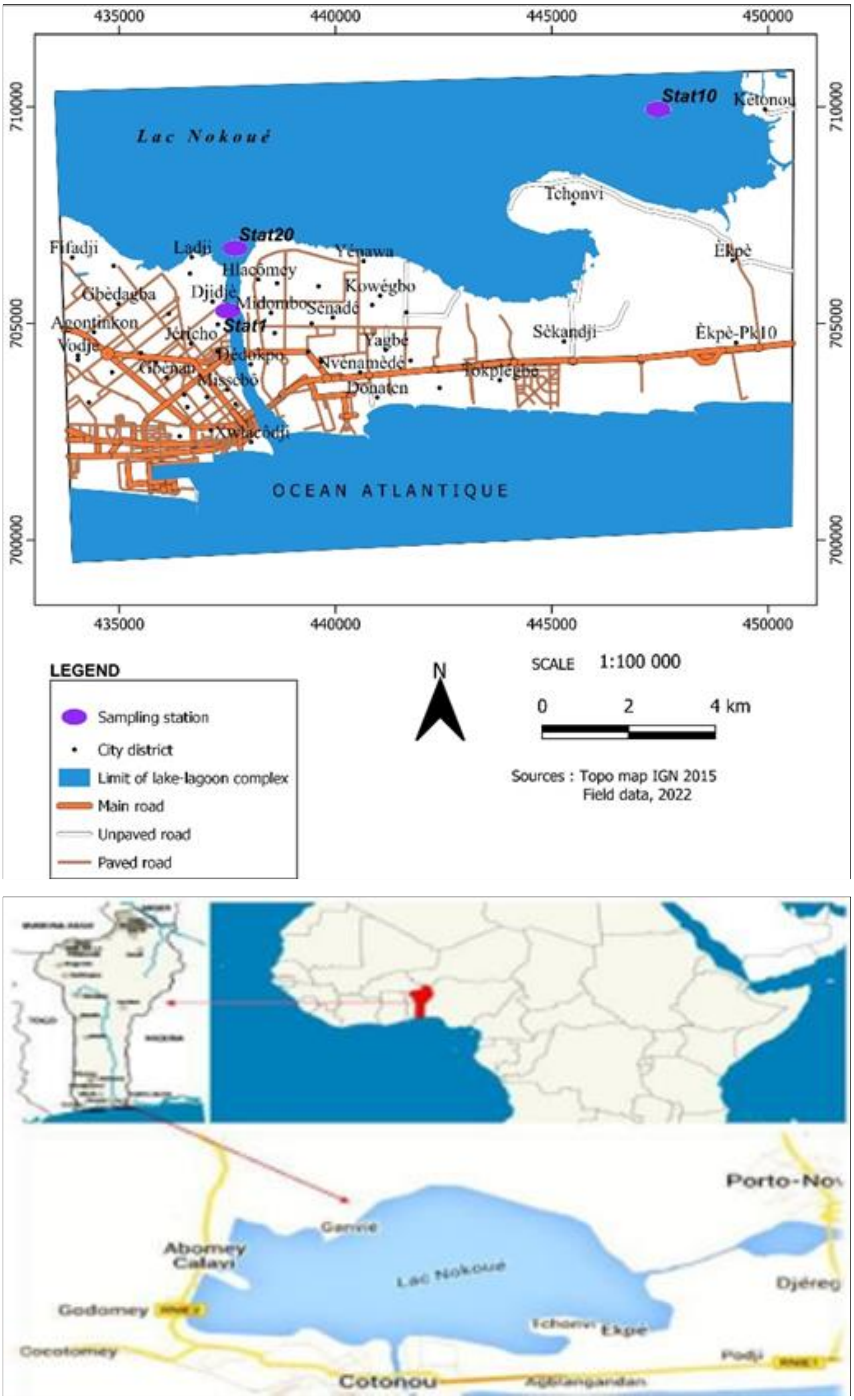

Fig 1: Presentation of the study area

The *Crassostrea species gasar* is exploited at Lake Nokoué by the Toffins peoples (Agadjihouede *et al*., 2017) [5] who have passed on this technique for several generations. It constitutes the first professional skill acquired by the operator, which provides them with substantial income or additional income for the fishermen. For the latter, oyster trading constitutes an alternative to the reduction of the ichthyological stock. The distribution of oysters on the lake is dependent on saline intrusion from the Atlantic Ocean via the Cotonou Channel. Some pockets of salinity are also observable at depth (Youssao *et al*. 2022) [9] in Lake Nokoué. The salinity at the surface is generally lower than that at depth. In general, fresh water overcomes marine waters which move with the tides and waves at depth. This difference in water distribution could explain the distribution of oysters, which are found more in moderately saline waters, because they are benthic species that live preferentially in brackish waters in contact with sediments from which they accumulate appreciable quantities of metals. Parameters such as temperature and salinity remain favorable for cupped oysters in Lake Nokoué in the month of December. The study area is made up of thirty-one (31) stations distributed in the Cotonou Channel and Lake Nokoué, but the points retained are the sites of Agbato, Djidjè and Ekpè due to the availability of oysters at these stations. Indeed, we questioned populations who carry out oyster harvesting as an activity and who live near Lake Nokoué. Questions about oyster trading at Lake Nokoué and areas of high

Sampling campaign

The sampling campaign took place in two stages. We first carried out an exploratory visit in order to choose the stations and then a sampling campaign during which the oyster samples and the measurements of physicochemical parameters were carried out. The exploratory visit made it possible to identify the sampling points by noting the various potential sources of pollution. Our choices are based on the facilities located on the lake (House, Acadja, latrines on stilts,) and the activities carried out in the area (landing of gasoline, fishing, oyster collection,). The habitat of the oysters is made up of a substrate of a sandy nature (at least 75% sand) in general, but the oysters cling to the wood of fish traps, installations on stilts and other solid waste discharged into the lake. Which are at the origin of a superficial mud above resulting from the processes of biodegradation of the latter. The sampled material consists of *Crassostrea* oysters *gazer* packed in plastic containers (zip bags). Indeed, the oyster is an edible lamellibranch marine mollusk, with an asymmetrical bivalve shell, coarsely laminated on the outside, pearly on the inside and which lives attached by its hollow valve to a submerged body. There are two forms of oyster namely: the cupped oyster and the flat oyster. The choice of this mollusc is justified by its great capacity to accumulate large quantities of heavy metals. What predisposes it to a role of bioindicator in the sediments of brackish waters contaminated by the residues and the metallic trace elements. For the sampling of oysters on Lake Nokoué we have requested the service of a harvester who ties around his waist the cord of a container and then goes down to the bottom of the water. Once in the water, he locates the colonies of oysters on the substrates with his feet and dives under the water. He then picks up the oysters with his hands, which he brings back to the container which floats on the surface of the water with its contents. Once the container is full, its contents are poured into the canoe. The oyster samples are packaged and then stored using cold accumulators at 4 °C until the laboratory where they are put in the freezer after measuring the size of the oysters.

Measurement of physico-chemical parameters of water

The hydrogen potential (pH), salinity, dissolved oxygen, conductivity and temperature of the water were measured at the surface using a digital device of the type Multi 3630 IDS. Our choice is motivated by the concern to analyze the variations due to the contributions of marine currents (via the channel) and continental ones coming in particular from the Ouémé River, the Sô and Djonou rivers. These periodic and seasonal variations condition the existence of oysters and therefore the collection activity by the toffin populations (Agadjihouede *et al*., 2017) [5].

Preparation of oyster samples in the laboratory

For metals analysis, adult (large) oyster samples were pooled to provide 150-200 g of tissue or approximately 20–30 shells (Farrell *et al*., 2018) [4]. To prepare the oyster samples for the assay, they were dried in a memmert UF brand oven at 40 °C. for 2 to 3 weeks, ground using a Retsch RM200 brand device. The actual dosage is carried out on a sample of 0.5 g of ground oyster in a flask to which 4 ml of pure sulfuric acid is added. The mixture is heated on the heating plate at a temperature of 500 °C. until stabilization of the medium. A few drops of hydrogen peroxide are added to clarify the medium and the whole is then cooled and then taken up with distilled water to reach a volume of 100 ml of mineralized.

A sample of 20 ml of the mineralized obtained was supplemented with distilled water until reaching 250 ml. To this volume we add a capsule of citrate buffers and leave to stand until the grains are completely dissolved in the mineralize. We then move on to making the mixture of 50 ml of Chloroform for a sachet of Dithiver. We poured the mineralize containing the buffer into a 500 ml capacity separating funnel and added the mixture of chloroform + dithiver to the mineralize.

To this mixture, we then added 5N soda and stirred vigorously until the color changed (pink colouring). Having obtained this coloration, we added 2 g of potassium cyanide then stirred vigorously until the second bend (orange coloration). We then place the separatory funnel on a suitable bracket and let the mixture rest in order to obtain the two phases, chloroform at the bottom and water at the top. We thus pass to the sampling of the chloroform phase in two test tubes by putting a little cotton in the orifice after the valve of the funnel to be decanted for the reading with the spectrophotometer.

Cadmium extraction

The difference with the Lead assay lies in the order of introduction of the reagents. We take 20 ml of the mineralize to which we complete distilled water up to 250 ml then add a capsule of Citrate Buffers. We then leave until the grains have completely dissolved in the mineralize. Meanwhile, here we make a mixture of 30 ml of Chloroform for a sachet of Dithiver. We make a mixture of 50% soda then 0.2 g of potassium cyanide in a volume of water of 10ml capacity which we shake vigorously. After stirring, we had to add the mixture of Chloroform + Dithiver and shake vigorously until the bend. We placed the separatory funnel on a suitable stem and left the mixture to stand (the two phases, chloroform at the bottom and water at the top, separate). We then put a bit of cotton in the opening of the separatory funnel valve to collect the chloroform phase in a tube while opening the valve slightly. After each shaking of the separatory funnel, you must degas through one of the openings to avoid an explosion. We then read the value with the spectrophotometer.

Lead mining

The method of carrying out mineralization is based only on waste water, surface water, residual water and all solids, except groundwater. It consists of taking 40 ml of waste water / taking 0.5 g if it is a solid or waste oil in a volumetric flask then adding 4 ml of pure sulfuric acid. Then, to heat on a hot plate at a temperature of 500 °C until stabilization of the medium then to add hydrogen peroxide drop by drop to clarify the medium. Now lower the flask and allow to cool before filling with distilled water to reach a volume of 100 ml.

Reading with a molecular absorption spectrophotometer

The molecular absorption spectrophotometer that we used in the laboratory is of the Photolab 6600 UV-VIS type. To do this, we made a blank with chloroform only to read the value on the spectrophotometer. We then introduce the tube containing the sampled chloroform phase and read the value of each tube. First of all, we have to carry out a blank with chloroform only and read the value on the spectrophotometer before any introduction of a tube containing the chloroform phase and read the value. Before any reading of the extracted chloroform phases, we carry out a blank with chloroform only for a preliminary reading of the value with the spectrophotometer.

Results and Discussion

Data collection is done through a field survey to qualitatively study the distribution of oysters on Lake Nokoué and a campaign of oyster sampling and measurement of physico-chemical parameters in the field. This follows a previous study in which it was not possible to obtain oysters for mercury analysis (Youssao *et al*., 2022) [9].

According to the results of our survey, 86% of those surveyed indicated that oysters (*Crassostrea gasar*) can be found in all areas of Lake Nokoué. The other 14% indicate that the east side of the lake which constitutes the entry point of the Ouémé River would be less rich if not devoid of oysters. The comments made by the respondents on the oyster fishing area indicate a proximity of the oysters to the Acadja which are types of fish traps made up of branches fixed in the sediments where the fish find freshness and a multitude of insects which proliferate there. The oysters cling to these woods used to constitute the fish traps to the detriment of the mangroves. The harvest takes place from March and can last four to five months; which corresponds to the end of the dry season and the beginning of the great rainy season in southern Benin. The wholesalers also indicate that after this period, the harvested oysters are often dead. Among the people surveyed, 97% indicate that the quantity of oysters harvested has not decreased over the years. These animals are therefore not threatened with extinction, but the risk at their level is sanitary. This result agrees with that of Morin. (2006) [10] indicating that these species can accumulate significant amounts of metals and that they could be used as bio indicators of heavy metal contamination of brackish water sediments. Taking into account the availability of a substrate and the optimal physico-chemical conditions for the oysters, we can propose the distribution map as shown in Figure 2 below. In this picture:

- The stations in orange (S_{12}, S_{14}, S_{16} and S_{24}) correspond to areas with optimal conditions for the development of oysters, not deep because not far from the banks but the activity is not really exercised because the area is deemed unsanitary. The substrate available in these areas is clinging to the latrines on stilts and the solid waste rejected by the occupants;
- The stations in white (S_{26} and S_{287}) are optimal areas but the activity is not carried out in these places. Oysters harvested from these locations are also hung on stilt constructions, leftover acadja wood, or other submerged solid waste;
- The stations in yellow (S_{20}, S_{27}, S_{29}, S_{30} and S_{31}) constitute the most important area for harvesting oysters on Lake Nokoué. It is not far from the banks and shallow;
- The stations in pink (S_{19}, S_{21}, S_{22}, S_{23} and S_{25}) are located in the center of Lake Nokoué. It is an area dominated by the establishment of acadja and there is no housing. The oysters at this level have a substrate made up of acadja but the depth of the lake at this level does not allow the harvesting of oysters.

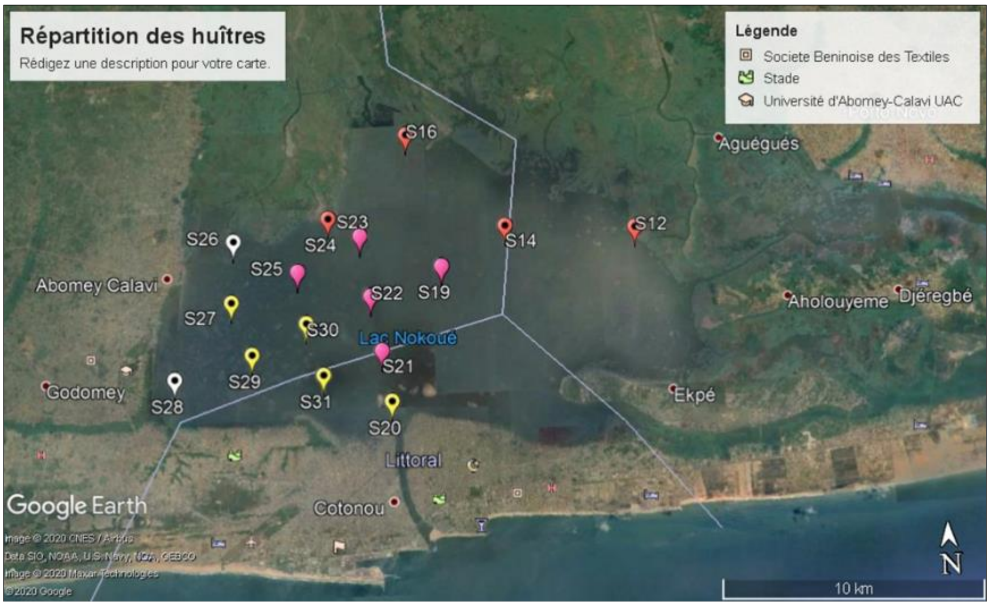

Fig 2: Distribution of oysters on Lake Nokoué

Physico-chemical characterization of lake waters

The graph in Figure 3 presents the results obtained after the physico-chemical characterization of the lake waters

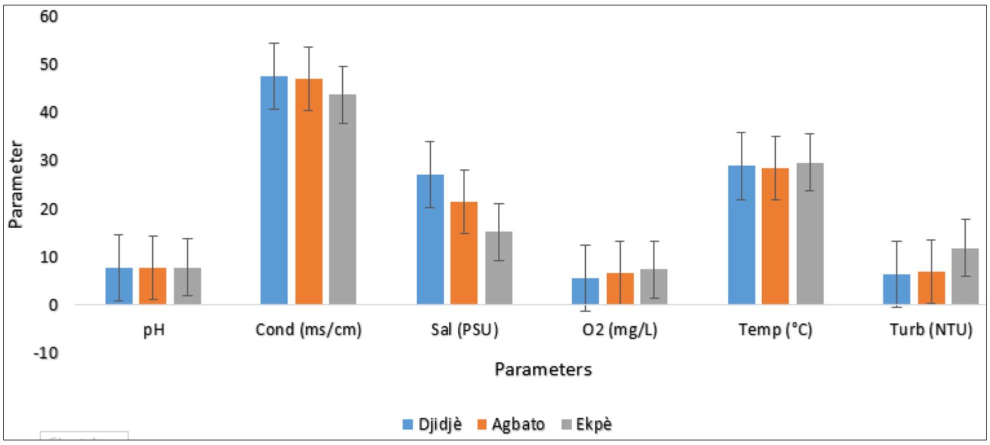

Fig 3: Physico-chemical parameters on the sites

The pH values recorded are constant and equal to 7.7 on the three different sampling sites. The measurements took place in the month of August during the short dry season in southern Benin. Compared to the aquatic development standards cited above, we can say that the lake remains a safe habitat for the species with regard to this parameter.

As for the conductivity, the highest value is 47.5 mS /cm (recorded in Djidjè) and the lowest value is 43.7 mS /cm (recorded in Ekpè) i.e. a difference of 3.8 mS /cm. It is therefore deduced from this that the contributions of dissolved substances are low at the level of the measurement sites.

Regarding salinity, there is a variation between 15.1 and 27.1 PSU. Thus, the highest value was recorded in Djidjè and the lowest value in Ekpè. The environment is therefore favorable to the development of oysters.

For dissolved oxygen (O_2), the values are between 5.5 mg/L (Djidjè) and 7.3 mg/L (Ekpè). Knowing that the standard in the aquatic environment for a better life of the species is 5 mg/L, we can conclude that the environment remains favorable for the oysters and the other species of Lake Nokoué.

The temperature varies between 28.3 °C (Agbato) and 29.5 °C (Ekpè), a variation of 1.2. This low variation is a favorable factor for the development of aquatic species in this ecosystem.

Overall, the water of Lake Nokoué is cloudy (5 < NTU < 30). The water at the Ekpè site is cloudier than the other two with a value of 11.7 NTU; it represents the maximum value and the minimum value is obtained at Djidjè (6.3 NTU). This can be explained by the different activities carried out on the banks of Ekpè such as fishing, breeding and market gardening. According to Širi´c *et al.* (2022) [21], locations near residential, industrial and transportation activities had higher levels of heavy metals and may increase metal accumulations in edible products.

Several studies have shown that the accumulation of metals by mussels is affected by salinity, temperature and pH of the water column (Biswas *et al*, 2013) [11] and that human interference and pollution of origin are also important factors in the migration of metals through the food chain (Hazell *et al*., 2021, Farrell *et al*. 2018) [12, 4]. The maximum fresh weight levels in molluscs, set for lead and cadmium at 2 mg/kg, authorized by the decree of 21 May 1999 are higher than the limits set by Regulation (EC) No 1881/2006 which are 1.5 and 1.0 mg/kg respectively. The results obtained, expressed in mg/kg of dry weight, are higher than the standards, but these values could be lower if they were related to the fresh weight. In general, lead concentrations are higher than that of cadmium except on the Ekpè site where the detection threshold is not reached for lead.

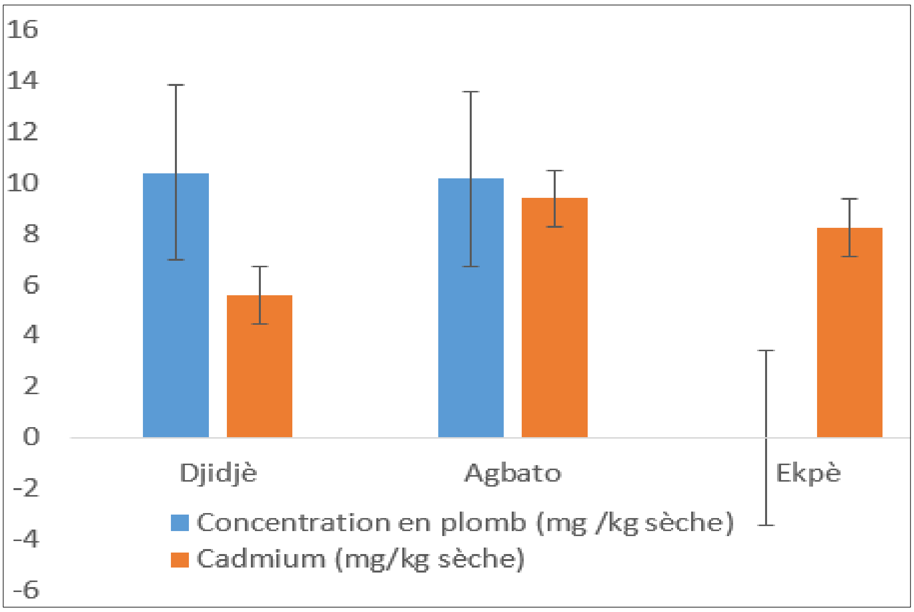

Fig 4: Comparison of Lead and Cd contents

The graph above (Figure 4) shows the lead and Cd contamination rates at the different sampling sites.

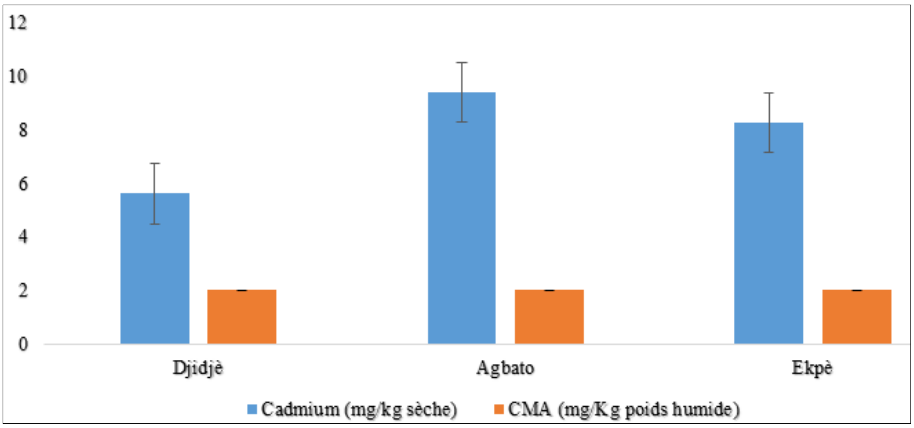

Fig 5: Comparison of Cadmium levels with regulations

The graph above (Figure 5) shows the cadmium contamination compared to the French standard. Note that this is generally higher than the standard indicated. The greatest value is obtained at site 2 (Agbato) and the smallest value at the Djidjè site.

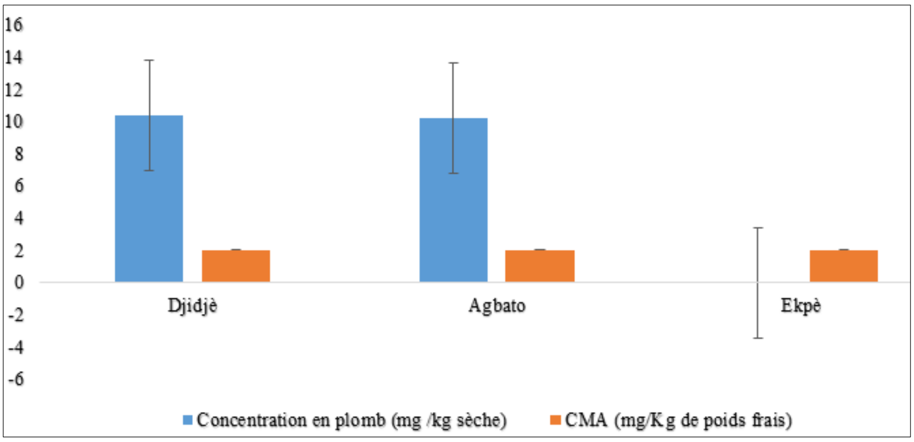

Fig 6: Distribution of metals on the sites

The graph above (Figure 6) shows the distribution of metals on the study sites. The contaminants are distributed heterogeneously and this can be explained by the different physicochemical conditions at the sites.

The lead concentrations recorded in the oysters collected on the Djidjè sites and Agbato are higher than the maximum concentration authorized by the French standard, which testifies to a contamination of the oysters of these environments in Lead. This contamination can be due, among other things, to the discharge of waste into the lake and to the discharge of water through the gutters that flow into it. Indeed, nowadays the shores of Lake Nokoué show wild dumps, receptacle for household waste, excreta, place of ease. They have become areas for handling pesticides and fertilizers linked to market gardening activities (Elegbede *et al*., 2020) [13]. The lead values observed on these two sites are relatively close (10.4 mg/kg for Djidjè and 10.17 mg/kg for Agbato). The Djidje website therefore seems more polluted with lead than that of Agbato. Lead pollution on the Ekpè site is below the detection threshold of the device. Various works carried out on Lake Nokoué and in the bodies of water in the study area have indicated contamination of sediments, water and living aquatic organisms. The values obtained are comparable but lower than those found by Elegbede *et al.* (2020) [13], on *Cardisoma* -like crabs *frame* from Lake Nokoué (35 mg/ kg). This difference can be explained by the fact that *Cardisoma* species *frame* have more affinity for sediments (Djèdjè, 2015, cited by Elegbede *et al.* (2020) [13]. Youssao *et al.* (2011) [7] indicated an average contamination of 130.77 mg/kg in the sediments of Lake Nokoué. Lead concentrations of 19.15 mg/kg were obtained in sediments collected at the Denou station in Nokoué by Goussanou *et al.* (2018) [14]. While the crab samples collected at Agonsagbo by these authors significantly recorded the highest concentrations of lead (1.13 mg/kg) and cadmium (0.23 mg/ kg). On the other hand, the highest cadmium concentrations (0.46 mg/kg) are obtained in the sediments collected at the Kétonou and Agonsagbo stations (Goussanou *et al*., 2018) [14].

All this could explain the lead concentrations obtained in the oysters on the different sites of this study. Indeed, oysters being benthic species, they feed on plankton and sometimes on the sediments on which they are fixed. The values noted in this study are all higher than those reported by Youssao *et al.* (2011) [7] and by Soumanou *et al.* (2012) [15] on shrimp from Lake Nokoué. These two species being benthic, it should be noted that oysters have a more intense mode of nutrition than that of shrimp. This could explain the difference between the values obtained.

Oysters are species that have aroused great interest for scientists around the world (Suam i *et al*., 2019; Ferreira *et al.* 2005, Hazell *et al.* 2021, Biswas *et al.* 2013, Castañeda - Chavez *et al*., 2014) [16, 17, 12, 11, 18]. Indeed, the invertebrates aquatic such that the molds and them Oysters, are used to estimate marine chemical and biological pollution due to their high metal accumulation capacity (Suami *et al*., 2019) [16]. Several studies have been carried out on oysters around the world. Ferreira *et al*. (2005) [17] reported concentrations of 0.8±0.18 and 0.13±0.11 mg.kg^{-1} fresh for Cd and Pb in *Ostrea Ostrea equestris* on the north coast of the state of Rio de Janeiro, Brazil. Hazel *et al*. (2021) [12] sampled oysters (*Crassostrea iredalei*) *for the analysis of heavy metals from three stations in* Cañacao Bay in the Philippines. Pb concentrations in oysters ranged from <0.1 to 0.4±0.1 mg/kg while Cd concentrations ranged from 0.027±0.006 to 0.083±0.006 mg/kg, but only Pb showed variations. Seasonal concentration. Suam i *et al*. (2019) [16] sampled oysters on the Atlantic coast of the Democratic Republic of Congo. The concentration in the oysters of Cd and Pb varied from 0.03 to 0.31 and 0.08 to 0.64 mg.kg^{-1} respectively. This result is still not with the lowest concentration of Cd obtained on the site of Ekpe which presents the lowest salinity (15.1 PSU) then it is the opposite that we observe for Pb. Biswas *et al*. (2013) [11] worked on the accumulation of cadmium and lead in the edible oyster, *Saccostrea cucullata* in coastal areas of West Bengal. Maximum concentrations of 37.01±1.442 and 31.369±1.266 mg.kg^{-1} were respectively obtained for cd and Pb at the Shankarpur and Satgelia stations (Biswas *et al*., 2013) [11]. The rate of absorption and accumulation of cadmium and lead by species oysters *Saccostrea echinata* was favored by of low salinities (Denton and Burdon Jones, 1981) [19].

Castañeda -Chavez *et al*. (2014) [18] studied the accumulation of heavy metals in oysters from lagoon systems in the southern Gulf of Mexico. The mean maximum Pb concentration was 321.15±28.828 mg.kg^{-1} and that of Cd was 63.74±8.446 mg.kg^{-1}.

Cadmium concentrations in oysters from Lake Nokoué are higher than the French standard and the values obtained by Suam i *et al*. (2019) [16], Ferreira *et al*. (2005) [17] and Hazell *et al*. (2021) [12]. But they are lower than those obtained by Biswas *et al*. (2013) [11], Denton and Burdon Jones (1981) [19], and Castañeda -Chavez *et al*. (2014) [18]. The maximum Cd value obtained on Lake Nokoué is 9.39 mg/kg PS (Agbato site) and the minimum value is 5.61 mg/kg PS (Djidjè site). The values obtained are close to those reported by Chouti *et al*., (2010) [20] who noted a cadmium concentration of 7.33 mg/kg at the level of the Totchè Canal, a communication point between Lake Nokoué and the Porto-Novo lagoon. According to Soumanou *et al.* (2012) [15], a maximum cadmium value of 0.15 mg/kg was recorded in shrimp from Lake Nokoué. This value is lower than that noted for oysters from Lake Nokoué. These are two benthic species, but the difference in accumulated lead concentration can be associated with their lifestyles and their diets. They are therefore more likely to accumulate high concentrations of heavy metals such as cadmium.

Conclusion

Dissolved oxygen is low at some stations, which could pose a threat to the life of aquatic species; which could be explained by the presence of landfills on the shore and in Lake Nokoué. In general, Lake Nokoué oysters are contaminated with lead and cadmium. Since these species are of great interest to populations, it is important to take action to protect them against the effects of human activity and the migration of these metals into the food chain via these benthic organisms. We can note, with regard to the high turbidity and the high Cd and Pb contents, that the physico-chemical conditions of Lake Nokoué are not favorable to the development of certain aquatic species such as oysters. However, the variations in pH, salinity and conductivity are within the limits of national and international standards and comply with the conditions necessary for the development of living aquatic organisms. The authorities must create the conditions to avoid the direct discharge of wastewater and municipal waste loaded into the gutters and bodies of water into Lake Nokoué by constantly monitoring the populations living along the rivers, lakes and the city of Cotonou.

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