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Heavy metal contamination of *Diplodus vulgaris* (Sparidae) in the Gulf of Annaba (North-eastern Algeria)

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ABSTRACT

The aim of this work was to evaluate heavy metals (Lead and Cadmium) contamination in the muscles of a local sea fish species, the black-headed Sar *Diploddus vulgaris*. We selected two sites in the Gulf of Annaba for this study (*Sidi Salem* fishing site and the Port of Annaba) and compared results to a reference site (*Cap de Garde*), which is at a valuable distance from any source of pollution, and is considered as a relatively clean site, while *Sidi Salem* and Port of Annaba are known to be exposed sites to various sources of industrial, urban and harbor pollution. Analyses have been carried out on 70 samples of dorsal-lateral muscles of fishes weighting from 30 g to 280 g and sizing between 11.1 cm to 26.5 cm. The results showed the presence of both metals in all samples during the 6 months of our study (from November to June) and in the 3 sites with variable rates. Cadmium levels varied from 0.032 to 0.239 mg/kg/fw. Fish meat from *Sidi Salem* collected during the months of November and February displayed a rate of 0.038-0.074 mg/kg/fw of Cd before reaching a higher value in March estimated to 0.202 mg/kg/fw of Cd. From April to June, the lateral dorsal muscles were contaminated by lower Cd contents. Pb concentrations varied from 0.014 to 0.149 mg/kg/fw. The highest value was recorded during the month of March at *Sidi Salem*. Moreover, Pb concentrations in muscle tissues increased significantly compared to the other studied sites. Very high significance differences were found between Cap de Garde and Port of Annaba and Sidi Salem for both metals (Pb and Cd). To conclude, the presence of heavy metals in *Diplodus vulgaris* is due to the diet and the impact of anthropogenic activities on the biotope of the fish and therefore poses a public health problem linked to frequent consumption of this fish. Also, *D. vulgaris* appears to be a suitable sentinel species for the assessment of ecotoxicological risk in the Gulf of Annaba.

Key words : *Diplodus vulgaris, Lead, Cadmium, Gulf of Annaba, Algeria*

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Introduction

Environmental contamination of food can take two forms: long-term, low-dose contamination, resulting from a gradual release of persistent chemicals into the environment and short term and higher concentration contamination, resulting from direct application, accidental release or contamination related to waste treatment (Panisset *et al.,* 2003). Heavy metals are among the harmful pollutants that arrive at sea (Ouali *et al.,* 2018; Tata *et al.,* 2020). This harmfulness is linked to their persistence, and because they are poorly metabolized, they can be transferred into the food chain and accumulated in living matter (Amirad, 2011). Fish is a valuable source of animal protein and other essential micronutrients including vitamins, minerals and omega-3 polyunsaturated fatty acids (FAO, 2014). Fish is an important part of the human diet and it is therefore not surprising that many studies are carried out on metal pollution in different fish species (Prudente *et al.,* 1997; Kucuksezgin *et al.,* 2001; Lewis *et al.,* 2002). Mediterranean fishes are exposed to various threats including pollution, habitat loss and human activities. Only a small percentage of species are threatened by invasive or exotic species (Golani *et al.,* 2010). However, several factors, especially abiotics ones (concentration of pollutant releases and physicochemical parameters of the environment) and biotics (species, sex, age, diet and metabolism) can affect metal accumulation in fish meat (Storelli *et al.,* 2005). Although fish muscles tend to accumulate low concentration of metals and it is important to check their security levels (Storelli *et al.,* 2006; Castro-Gonzalez *et al.,* 2008; Zhuang *et al.,* 2013; Ben salem *et al.,* 2014). Metallic Trace Elements (MTE) such as Mercury, Cadmium and Lead are persistent inorganic pollutants representing a group of toxic substances which are transferred along the food chain to humans (Storelli, 2008). On the eastern coast of Algeria, the fishing activity is more akin to the artisanal. Coastal fishing mode and targets many species of Teleostean fishes of economic interest. Among these, the Sparidae family constitute one of the best represented families (Dieuzeide *et al.,* 1953; Derbal *et al.,* 2001) with 10 genera and 24 species colonizing coastal and offshore waters, on very varied backgrounds (rocks, sands, and sea grass). In the family of Sparidae, Sars of *Diplodus* genus are of great socio-economic importance. *Diplodus vulgaris* is a teleostean fish that lives in coastal waters, of rocky or sandy bottoms, up to a depth of 130m (Fisher *et al.,* 1987). Diplodus vulgaris is a littoral demersal species whose juveniles live on sea grass funds (El-Morhit *et al.,* 2013). The demersal species and benthopelagic species are closer to the sediments which are often considered as reservoirs of many chemical pollutants, especially trace elements (Yao *et al.,* 2009; Chouti *et al.,* 2010). Diplodus vulgaris is a sentinel species considered to be a good bio-indicator of aquatic ecosystems quality (El Mohrit *et al.,* 2008). The aim of our work is to evaluate the metal contamination (by Lead and Cadmium) in *Diplodus vulgaris* sampled in the Gulf of Annaba.

Materials and Methods

Sampling sites

Samples were collected in three sites located in the Gulf of Annaba (*Cap de Garde*, fishing Port of Annaba and *Sidi Salem* fishing area). Annaba Gulf is an enclave of the Mediterranean Sea. It is located in the far northeast of Algeria (Figure 1). The gulf of Annaba is bounded to the west by *Cap de Garde* (57°16′E; 36°58′N) and to the east by *Cape Rosa* (8°15′E; 36°58′N). The maritime frontage of this zone extends over a length of approximately 40km of coasts representing a very important halieutic potential.

- The *Cap de Garde* considered in this study as a reference site is located in north of Annaba Gulf (36°57′54.00"N; 7°47′27.99"E) and its water receives western currents. The area is considered to be a witness in view of the absence of major sources of pollution (limited urbanization, no industrial plant, no agricultural activity…etc.).
- The fishing port of Annaba is located in the heart of the bay (36°54′21.30"N; 7°46′34.78"E) and completely landlocked. This port receives different sources of pollution due to the economic activity of the port. The port of Annaba is one of the ten main commercial ports of Algeria.
- The *Sidi Salem* fishing area is located south of the city of Annaba (36°51′28.28"N; 7°47′28.83"E). It is backed by an industrial area (agricultural fertilizer production industry). It also receives the discharge water from *Oued Seybouse*, one of the most important rivers in the east of Algeria.

Biological material

During our present study, we were interested in a

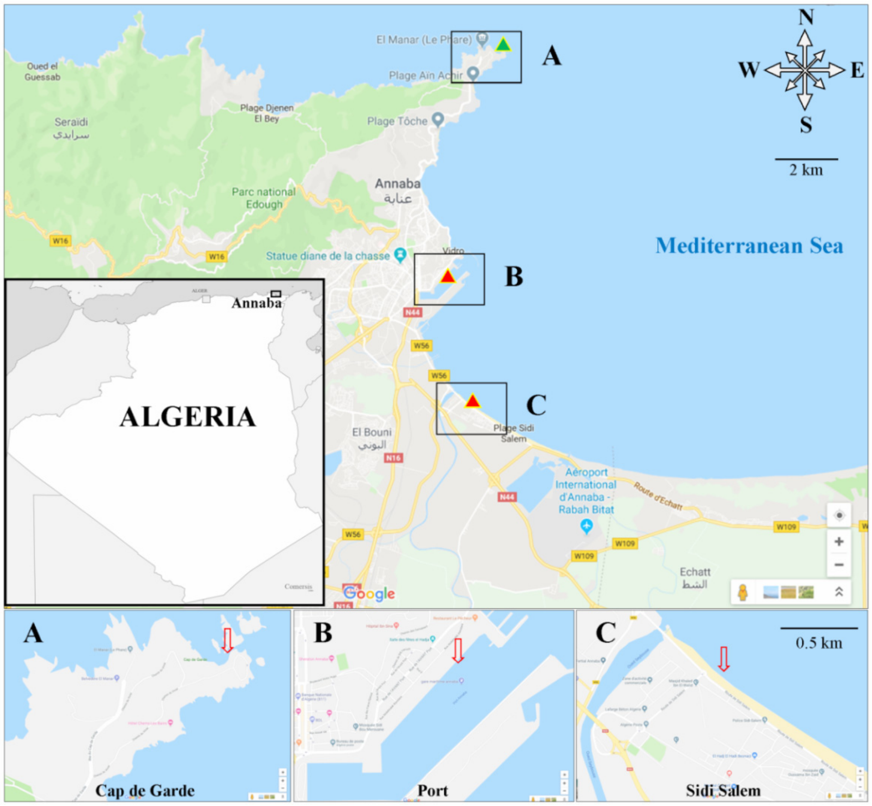

Fig. 1. Sampling sites from the Gulf of Annaba (North-east, Algeria)

species consumed locally, the black-headed Sar: *Diplodus vulgaris*, belonging to the Sparidae family. Fishes were collected during a period of six (06) months at the rate of five (05) individuals per month and per site. The objective was to determine the metal contamination thresholds by assaying the concentration of toxic non-essential heavy metals, Lead and Cadmium, in the muscle of the fish. Sar fish samples were then cleaned, gutted headless to keep the skinless dorsal muscle for metal analysis, according to De Souza *et al.* (2002). The study of contaminants and metallic trace elements in fish is often focused on muscle in order to study the transfer of these elements to human populations via their diet. Samples were placed in closed and labeled plastic bags; the label includes the code, the date of sampling and the sampling site. The bags were then transported to the Biochemistry and Environmental Research Laboratory (Department of Biochemistry at Badji Mokhtar Annaba University) for their storage at -20 °C.

Determination of heavy metals

Samples of back muscles from the five fishes were first crushed, mixed and oven-dried at 80 °C for 48 hours. After drying, each sample was finely grounded. Obtained powder was well homogenized and putted in polyethylene flasks for storage in a dry place. Wet mineralization was applied to samples, where 5 ml of nitric acid and 2 ml of hydrogen peroxide H_2O_2 have been added to 0.5 g of powder, the whole mixture was heated in an incubator at 105°C during two hours. The mineralized solutions were filtered and stored at +4 °C in labeled polyethylene flasks until assessment of heavy metals concentration. The dosage of Lead and Cadmium was carried out at 520 nm with UV-visible spectrophotometer Hach DR 3900.

Statistical analysis

The obtained data were expressed as mean ± standard deviation (SD). To make comparisons between samples sites, we used MCA (Multiple Correspondence Analysis), Student and Anova tow-ways tests. For all tests, the significance level for differences in critical values was set to p-value 0.05. All statistical analyses were performed using R (version 4.0.1; R Development Core Team 2020).

Results

Samples of *Diplodus vulgaris* weighted from 30 to 280 g and sized between 11.1 cm to 26.5 cm. The adult black-headed *Diplodus vulgaris* has generally a length between 15 cm and 30cm. The largest can reach 45cm (Geoffroy Saint-Hilaire, 1817).

Results of our investigations showed globally the presence of Lead (Pb) and Cadmium (Cd) in all samples (Figure 2) of *Diplodus vulgaris* with important amounts regarding to E.U. standards (Table 1). Statistical analysis revealed high variations

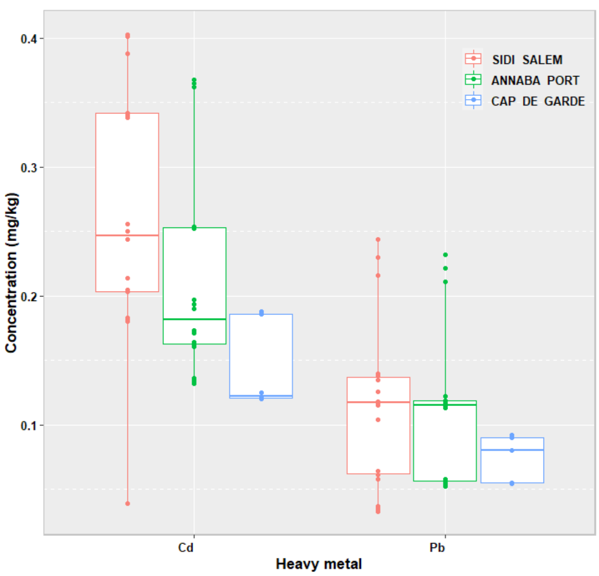

Fig. 2. Heavy metals levels in fresh weigh of *Diplodus vulgaris* regarding to EU standards

($p<0.0001$) between sites and months during the six months at the three sites (Table 1).

The rates of Cadmium varied from 0.1 to 0.15 mg/kg/fw during the first three months of sampling (November, February, and March) at *Sidi Salem*, from 0.1 to 0.4 at the fishing Port of Annaba and from 0.03 to 0.04 in the control site (*Cap de Garde*). These last rates of the control site were lower than those of the two previous sites (Figures 3). We observed in *Sidi Salem* fishes sampled during November and February 0.1mg of Cadmium/kg/fw before reaching a higher value in March (0.15mg/kg/fw). This value is higher than the limit (0.1 mg/kg/fw) set by the European Community (2002) for the muscle meat of black-headed Sar (*Diplodus vulgaris*). We also noticed a significant difference between *Cap de Garde* (reference site) and polluted ones (Annaba Port and *Sidi Salem*) (Table 1; Figure 3).

From April to June the lateral dorsal muscles of fish samples were contaminated with lower Cd rates 0.057 mg/ kg/fw in the control site; from 0.038 to 0.202 in the port of *Sidi Salem* and from 0.032 to 0.051 mg/kg/fw in the Port of Annaba. These rates remain below European standards**.**

Overall the samples of *Sidi Salem* contain higher amounts of Cadmium than those from the fishing Port of Annaba, even more than the control site *Cap de Garde* (Figure 2). Amounts of Cd found in the muscles of the same species in Moroccan Atlantic coasts reached 0.01 mg/kg/fw from *Foum l'Oued*, 0.06 mg/kg/fw and0.09mg/kg/fw at Laâsilia (Morhit *et al.*, 2013). These results reflect how the

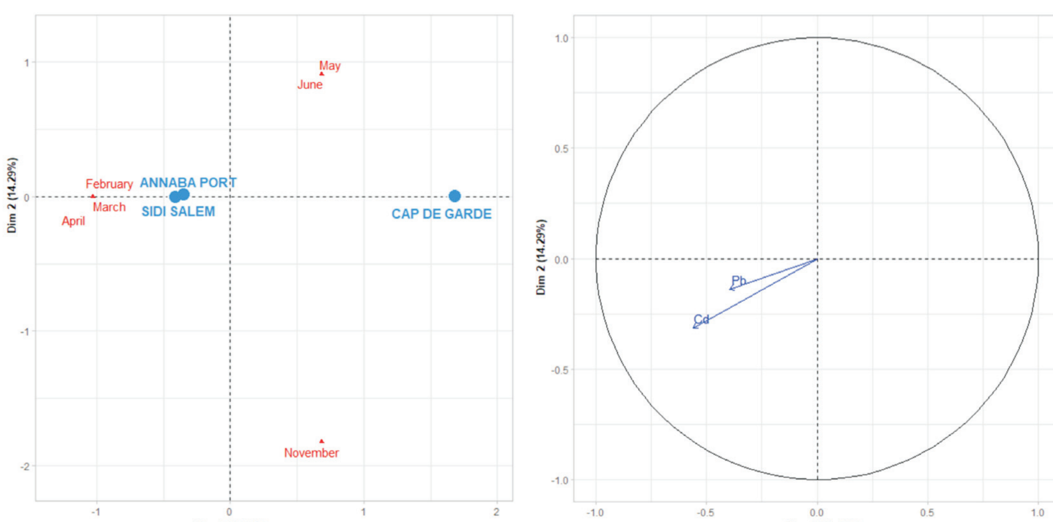

Fig. 3. Multiple Correspondence Analysis of heavy metals levels of *Diplodus vulgaris*

high level of pollution of the biotopes can affect this fish species. This is probably linked to the proximity of *Sidi Salem* sampling site to diverse acute pollution sources (many nearby factories).

Lead (Pb) metal was also present in all the dorsal lateral muscles of *Diploddus vulgaris* analyzed in our study. Overall, the black-headed Sar was slightly contaminated by this metal. The Pb values varied from 0.001 mg/kg/fw, the lowest value recorded at the control site (*Cap de Garde*) during the month of April to 0.14 mg/kg/fw, highest value measured during the month of March at the port of *Sidi Salem.* Thus all the rates recorded were below the European Standard which requires 0.4 mg of Pb /kg per fresh weight.

We also note that the muscle tissue from *Sidi salem* displayed higher lead/kg/fresh weight concentrations compared to other samples from the other studied sites (Figure 2). Except in the samples for the month of February, where we observed a lead rate at Port of Annaba, higher level in the order of 0.05 mg/kg/fw compared to that of *Sidi salem* at 0.02 mg/kg/fw.

Discussion

Presence of heavy metals is due to the diet pattern of this species and the contamination of its biotope (Tata *et al.,* 2020). Indeed, *Diplodus vulgaris* juveniles live on sea grass funds (El-Morhit *et al.,* 2013) and closer to sediments considered as reservoirs of metallic trace elements (Yao *et al.,* 2009; Chouti *et al.,* 2010). According to Ramade (1979), in teleostean fishes, metallic elements are particularly concentrated in the liver but also in the kidneys and weakly in the muscles. *Oued Seybouse* river suffers from anthropogenic pollution of multiple origins. These waters receive directly industrial and urban discharges (Belhamra *et al.,* 2016). Several studies have been carried out on surface waters as well as on sediments of *Oued Seybouse*. Hammadi *et al.* (2010) found that the concentration of heavy metals in water of surface greatly exceed the standards of the European Union (1998). Louhi *et al.,* (2012) reported in their work that the average concentration of metallic elements exceed acceptable standards for heavy metal pollution of sediments. The fishing Port of Annaba is one of the ten main commercial ports of Algeria. The rate of total hydrocarbons also recorded in this water (111mg/l) exceeds the standard of the Algerian Official Journal (Feknous *et al.,* 2017). According to Belabed *et al.* (2013) the chemical pollution of the industrial-port area of the Gulf of Annaba by heavy metals shows a worrying degree of contamination; result of port activity and direct exposure to anthropogenic effluents. Another study by Ouali *et al.,* (2018) in the Gulf of Annaba has shown alarming contamination by these two trace metals (Cd and Pb) in the sediments and in the tissues of another species of fish, the gray mullet (*Mugilus cephalus*). Belabed *et al.* (2013; 2017) studies have listed many heavy metal pollution sources in the Gulf of Annaba (Mechanical production site, cement plants, glass factory, paper mills, plastics and biomedical manufactory, battery recycling, phytosanitary factory…etc.) and demonstrated that the Steel industries is the main one heavy metal pollution sources (Arcelor Mittal, El Hadjar, Ferovial). Ouali *et al.* (2018) also demonstrated that both studied sites contain high amounts (mg/l) of Lead (Port of Annaba 272.54 ± 77.39; *Sidi Salem* 120.63 ± 45.14) and Cadmium (Port of Annaba 3.20 ± 0.88; *Sidi Sa-*

Table 1. Comparisons of heavy metals levels of *Diplodus vulgaris* in the Gulf of Annaba

Heavy metal	mean ± SD	Min	Max	IC	Student test			EU
					Df	t	P	Standards
Pb	0.11 ± 0.06	0.033	0.24	[0.08-0.12]	44	-34.95	0.0001***	0.4
Cd	0.22 ± 0.09	0.039	0.403	[0.19-0.25]	44	8.66	0.0001***	0.1

Factors	Heavy metals	ANOVA two-ways (n=3)		
		Df	F	P
Sites	Pb	2	177.6	0.0001***
Months		5	603.7	0.0001***
Sites x Month (interaction)		7	138.3	0.0001***
Sites	Cd	2	22.15	0.0001***
Months		5	11.42	0.0001***
Sites x Month (interaction)		7	7.91	0.0001***

lem 1.79 ± 0.22). These results were correlated with trace metal concentrations (mg/kg) in the muscle of *Mugil cephalus* for both metals, Lead (Port of Annaba 2.4 ± 0.08; *Sidi Salem* 1.23 ± 0.04) and Cadmium (Port of Annaba 0.95 ± 0.025; *Sidi Salem* 0.532 ± 0.03). On the other hand, because of the toxicity of Cadmium and Lead even at low concentration, as well as classified as contaminants priority by the European Union (Haynes and Johnson, 2000; Mcpherson and Chapman, 2000; Hagopain Schlekt *et al.*, 2001). The danger of this metal pollution in the aquatic environment lies in the toxicological risk which can be induced during the consumption of these products, hence its direct impact on human health (Amiard *et al.*, 2011). These pollutants can be bio accumulated and bio amplified, in the food chain and consequently at certain thresholds they become dangerous. Indeed, heavy metals tend to accumulate in consumers of the upper trophic level in the food chain (Kamilou *et al.*, 2014).

Cadmium

Cadmium is a highly toxic and ecotoxic non-essential metal (Stancheva *et al.*, 2013). It has no known metabolic role and does not appear to be biologically essential or beneficial to the metabolism of living beings (Miquel, 2001). Cadmium is a cumulative toxicant with a biological half life of around 20 to 30 years. Chronic exposure to this metal leads to the appearance of an irreversible nephropathy which can progress to renal failure (Bisson *et al.*, 2011). The main origin of Cadmium in coastal environments comes from the leaching of agricultural land containing fertilizers (Nriagu and Pacyna, 1988). The waters of this Gulf receive in the East zone the discharge from the *wadis* (rivers): Seybouse and Mafragh transporting terrigene, agricultural, domestic and industrial waters from the regions of Guelma, El-Tarf and Annaba. *Diplodus vulgaris* feeds specially on shellfish, mollusks, and even sea urchins. It has robust molars teeth capable of breaking shells (Husson, 2016), known for their ability to concentrate metallic trace elements. In general, higher concentrations of Cadmium are found in meat and viscera of crustaceans, mollusks and cephalopods (Miquel, 2001). Cadmium mainly accumulates in the hepatopancreas of crabs and Cadmium concentrations of 30-50 ppm have been detected in this animal. Cadmium usually bio-accumulates in the liver and kidneys of adult animals. These two organs, consumed in certain animals and fishery products, are the most important food sources of metal trace elements (Goulding, 2016).

Lead

Our Lead results are similar to those found in the same fish by Morhit *et al.* (2013) on the Moroccan coast, reaching 0.64 mg/kg/fw at *Foum l'Oued*; 0.038 mg/kg/fw and 0.007 mg/kg/fw at Laâsilia. Even, if Lead amounts are considered low, the presence of this non-essential metal in muscle tissue can have health consequences. Pb contamination is mainly attributed to urban and industrial liquid discharges, as well as to the pollution generated by automobile traffic (Bustamante *et al.*, 2003).

Conclusion

A total of 70 individuals of *Diplodus vulgaris* were collected from the Gulf of Annaba, with an average size of 11.1 cm to 26.5 cm and a weight of 30 to 280gr. The results of analyzes showed the presence of Lead and Cadmium in all the samples of fish muscle tissue taken during the 6 months of our study at the 3 sites with variable rates. Leads rates varied from 0.1 to 0.15 mg/kg/ fw during the first three months of sampling at *Sidi Salem*, from 0.04 to 0.1 at the Port of Annaba and from 0.03 at 0.04 at the *Cap de Garde* control site. Collected fishes from *Sidi Salem* during the months of November and February displayed a rate of 0.1mg/kg/fw before reaching a maximum value in March estimated at 0.15mg/kg/fw. From April to June the lateral dorsal muscles were weakly contaminated with Cd rates ranging from 0.001 to 0.014 mg/kg/fw in the control site, from 0.017 to 0.024 in the port of Sidi salem and from 0.006 to 0.016 in the Pot of Annaba. Pb was also present in all *Diplodus vulgaris* muscles with low concentrations. The Pb values varied from 0.001 lowest value recorded at the control site (*Cap de Garde*) during the month of April to 0.14 highest value measured during the month of March at the port of *Sidi salem*. The muscle tissue taken from the fishing port of *Sidi Salem* showed the highest lead kg/fw concentrations compared to other samples from the other sites studied, except in the samples of February where a lead level was observed at Port of Annaba higher on the order of 0.05 mg/kg/fw compared to that of *Sidi Salem* which was at 0.02 mg/kg/fw. The presence of heavy metals in *Diplodus vulgaris* taken from the northeast coast of Algeria is due to the diet and the impact of anthropogenic activities on the biotope of the fish studied at the two sites and therefore poses a public health problem linked to frequent consumption of this fish. In order to guarantee food security for the consumer, these fishery resources must be preserved by setting up industrial waste treatment stations to avoid toxicological contamination of the sea and sea products.

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Disclosure Statement

The authors report no conflict of interest. The authors alone are responsible for the content and writing of the article.

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