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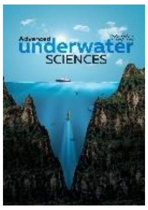

Determination of Effects of Seasonal and Sampling Area on Ulva Rigida's Elemental Composition

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ABSTRACT

Macroalgae is used as a bioindicator for their accumulation capacity of potentially hazardous elements. Ulva spp. was used extensively in previous studies as in the recent study. Along the Mersin coast, sampling was made in four different locations (Çamlıbel Marina, Pozcu Marina, Deniz Feneri, Karaduvar) during the spring and summer seasons between 2015-2016. For elemental analyses, ICP-MS was used, and Mg, Al, K, Ti, Cr, Mn, As, Se, Sr, Mo levels were analyzed in Ulva rigida samples. The highest level (134960.0 µg g-1) was found in the elemental analysis of Mg, and the lowest levels (20.83 µg g-1) were found in the elemental analysis of Se. Potentially hazardous trace elements (Al, Ti, Cr, Sr, As, Mn) were found in high levels in all seasons and sampling points in comparison to other studies. The abundance in other elements (Mg, K, Mo, and Se) was also notable in the same sense. The seasonal difference seems to have little effect on the accumulation of trace elements for our study. Pozcu Marina and Karaduvar stations are good sampling points in regard to monitor potentially toxic elements in macroalgae tissue.

1. INTRODUCTION

Macroalgae is used as a term for seaweeds and other benthic marine algae that can be seen by the naked eye. Although they are not really "weeds", larger macroalgae are also referred to as seaweeds (Diaz-Pulido and McCook, 2008). Seaweeds are abundant in the aquatic environment, they are sedentary and can be easily collected and identified (Campanella et al., 2001). They are mostly distributed in the rocky intertidal zone in most of the marine environment, therefore seaweeds are very important ecologically (Murphy, 2007). Based on their chemical composition, macroalgae are classified into three divisions: green (Chlorophyta), red (Rhodophyta), and brown (Phaeophyceae) algae (Gupta and Abu-Ghannam, 2011). Ulva genus is one of the green macroalgae and they grow in shallows waters and are specifically found in the marine environment (Loughnane et al., 2008).

Ulva sp. contain protein, essential fatty acids, minerals, polysaccharides, carotenoids, etc. in their structure (Fleurence, 1999). Environmental (salinity, temperature, pH, season, etc.) and biological (age, thallus morphology, etc.) factors can affect the accumulation levels of these elements (Lobban and Harrison, 1997). In addition, it is known that several macroalgae can accumulate high levels of metals and they can grow in the coastal waters which contain high levels of metal levels (Pawlik-Skowronska et al., 2007). Macroalgae can indicate pollution in the marine environment thanks to their characteristics such as (i) most of them are sessile; (ii) they are widely distributed and available all year round; (iii) they can tolerate wide ranges of salinity, turbidity, and high levels of pollutants; (iv) they are easy to collect and process; (v) they can be kept in laboratory conditions (Haug et al. 1974; Phillips 1990).

Macroalge's usage of bioindicators has started in the early 1950s, and mainly it was in UK and Canada at the beginning (Black and Mitchell 1952; Fuge and James 1973; Wort 1955; Bohn 1975). Because of its easy implantation today, it is used worldwide. It can be either native (passive biomonitoring) or transplanted species (active biomonitoring) (Garcia-Seoane et al. 2018).

Some metals such as Cu, Fe, Zn, Cr, Mn, etc. are essential for the human body to continue its functions. However, most heavy metals such as Cd, Pb, Hg, As, Cr,

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etc. are dangerous substances for they are harmful to human metabolism. Because they are toxic, non-biodegradable, have a very long half-life in soil (Singh et al., 2011). Heavy metals can accumulate in living systems through the active food chain.

In Mersin coastal zone, Alp et al. (2012) have investigated some of the heavy metal levels (Al, Cr, Mn, Fe, Ni, Cu, Zn, Pb, Cd) in Ulva sp. and Enteromorpha sp. and in sediment. Furthermore, Altun (2017) has also investigated some of the heavy metals (Fe, Cu, Cd, Zn, Pb) in the Mersin coastal zone.

Although there were several studies in the past years about heavy metal levels that accumulated in macroalgae in Mersin coastal area, the elemental composition of macroalgae may change constantly over the years. Furthermore, previous studies have concluded that the studies about the heavy metal levels in macroalgae should continue to cover reliable information to the researchers and authorities and thus, there are several macroalgae species to be investigated in this regard. The aim of this study was the determination of the elemental composition of Ulva rigida regarding the seasonal changes and sampling area.

2. MATERIALS AND METHODS

2.1. Collection of samples

The study was carried out in Mersin which has the largest port in Turkey. The territorial border of Mersin is 608 km with an area of 15.953 km2 and the sea border is 321 km. The study was carried out by sampling monthly, between April 2015 and May 2016 at the coastal stations, which were selected in different parts of Mersin province's coastline. The stations were as follows; Çamlıbel Marina (36°47' 27.6"N 34° 37' 36.2"E), Pozcu Marina (36°46' 06.1"N 34°34' 00.5"E), Deniz Feneri (36°47'05.0"N 34°37'15.8"E), and Karaduvar (36°48'27.1"N 34°41'31.4"E). The sample stations were shown in Figure 1. During the study, the samples are taken from the littoral zone.

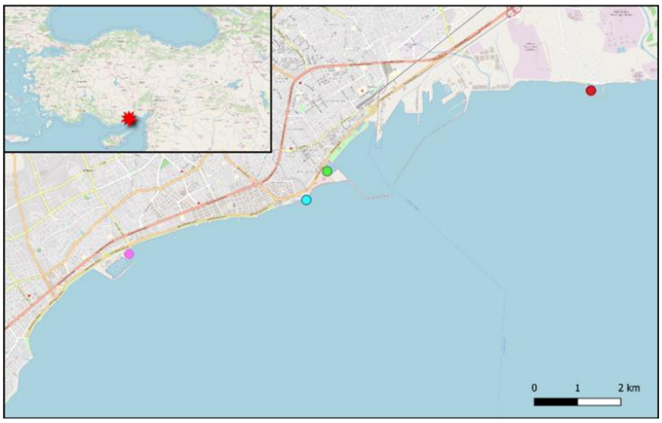

Figure 1. Sampling Stations in Mersin Bay (Pozcu marina purple, Deniz feneri as cyan, Çamlıbel marina as green, Karaduvar as red)

2.2. Storage and preparation of samples for analysis

The samples were put into polyethylene bags and labeled to indicate the date and stations. They were washed to remove the sand and other materials then dried at room temperature.

To make the samples dried, the samples were put in an incubator at 70 °C for 2 hours. After the drying process was done, the samples were shredded into little pieces with the shredder and again put back to the polyethylene bags. They were kept at +4 °C in a refrigerator until the time that they will be analyzed.

2.3. Elemental analysis

In this study, ten elements that accumulated in U. rigida's tissue were analyzed; those were Mg, Al, K, Ti, Cr, Mn, As, Se, Sr, and Mo. For that purpose, the samples were made soluble. From each U. rigida sample, 0.1 g was taken. 4 ml HNO_3, 2 ml $HClO_4$, 2 ml H_2O_2, and 2 ml H_2SO_4 were added to each sample's tube, and then they were heated on the hot-plate until they become homogenized. Then dilutions and pH adjustments of the samples were made. The samples were diluted in a ratio of 1:100 and the analysis was made via inductively coupled plasma mass spectrometry (ICP-MS, 7500-Ce) at the Mersin University Advanced Technology Education, Research and Application Center (MEITAM).

2.4. Statistics

Prior to the analyses, all data were checked for outliers, and homogeneity of variance was also tested. Statistical analysis of data was carried out with the IBM SPSS STATISTICS 22 statistical program. ANOVA (Analysis of Variance) was used to evaluate the effect of seasons and stations on the elemental profiles.

3. RESULTS

Maximum, minimum, and mean values of elemental levels and statistical differences between seasons and stations were presented in Table 1. Mg (182000.0 µg g-1) is the most abundant element, and it was at Pozcu Marina station in summer, Se (20.26 µg g-1) was the least found element in the structure of U. rigida and it was found at Deniz Feneri station in summer.

The highest level of Mg (182000 µg g-1) was found at Pozcu Marina in the summer season, while the lowest level of Mg (37002.72 µg g-1) was found at Karaduvar station in spring. There is a statistically significant difference between Çamlıbel Marina, Deniz Feneri, Karaduvar, and Pozcu Marina stations for the samples both in the spring and summer seasons. The highest and lowest levels of Al (2882.47 µg g-1 - 230.69 µg g-1, respectively) were found at Çamlıbel Marina in spring. There is no statistically significant difference between stations, both in the spring and summer seasons.

Table 1. The effects of season and sampling area on element levels of *U. rigida* (µg g^{-1})

	Çamlıbel Marina $\bar{X} \pm S_{\bar{X}}$	**Pozcu Marina** $\bar{X} \pm S_{\bar{X}}$	**Deniz Feneri** $\bar{X} \pm S_{\bar{X}}$	**Karaduvar** $\bar{X} \pm S_{\bar{X}}$	**Season**
Mg	67544.0±2792.24^{x} (54501.99-78240.47)	105420.0±25011.1^{y} (62104.23-149000.00)		37119.0±66.95^{x} (37002.72-37234.66)	**Spring**
		134960.0±27448.5^{y} (87413.33-182000.00)	59745.0±716.60^{x} (58503.40-60985.78)		**Summer**
Al	1297.5±340.05^{x} (230.69-2882.47)	1087.5±362.18^{x} (460.18-1714.83)		1570.1±71.67^{x} (1445.96-1694.26)	**Spring**
		1651.6±265.88^{x} (1191.11-2112.15)	663.47±29.57^{x} (612.24-714.69)		**Summer**
K	32181.0±1707.32^{y} (23440.86-39840.64)	44746.0±9915.66^{z} (27571.29-61920.15)		36383.0±176.98^{yz} (36076.29-36689.39)	**Spring**
		11691.0±2295.37^{x} (7715.56-15666.95)	8026.0±28.76^{x} (7679.19-8075.83)		**Summer**
Ti	1183.1±34.28^{x} (1053.20-1342.86)	1931.5±423.70^{y} (1197.64-2265.40)		1201.3±3.49^{x} (1195.28-1207.40)	**Spring**
		1923.6±397.26^{y} (1235.56-2611.72)	1159.6±52.87^{x} (1068.03-1251.18)		**Summer**
Cr	97.18±5.72^{x} (77.20-123.84)	176.41±43.36^{y} (101.30-251.52)		95.96±4.93^{x} (87.41-104.52)	**Spring**
		195.65±41.06^{y} (124.52-266.78)	100.17±5.14^{x} (91.26-109.08)		**Summer**
Mn	95.32±18.12^{x} (32.65-179.39)	105.9±25.81^{x} (61.20-150.61)		88.61±2.63^{x} (84.05-93.18)	**Spring**
		224.08±47.21^{y} (142.29-305.86)	129.44±7.39^{x} (116.63-142.26)		**Summer**
As	59.51±14.09^{x} (20.44-157.51)	34.21±7.36^{x} (21.46-46.98)		127.24±34.66^{y} (67.21-187.28)	**Spring**
		60.63±7.26^{x} (48.05-73.22)	23.66±0.53^{x} (22.74-24.59)		**Summer**
Se	65.40±21.29^{x} (24.89-220.62)	22.24±0.34^{x} (21.65-22.83)		181.43±62.42^{y} (73.30-289.55)	**Spring**
		35.16±2.22^{x} (31.32-39.02)	20.83±0.33^{x} (20.26-21.42)		**Summer**
Sr	177.79±19.05^{x} (86.31-289.14)	238.83±60.78^{x} (133.55-344.11)		123.36±4.59^{x} (115.40-131.31)	**Spring**
		453.08±120.14^{y} (244.98-661.17)	255.89±24.31^{x} (213.78-298.01)		**Summer**
Mo	188.65±47.48^{xy} (53.00-525.43)	82.32±0.16^{x} (82.05-82.60)		330.10±95.41^{y} (164.83-495.37)	**Spring**
		84.58±4.24^{x} (77.23-91.95)	130.08±8.40^{x} (117.00-154.77)		**Summer**

Different letters (x,y,z) in the same rows and columns for each metal significant differences ($p<0.05$). : mean±standard error; () shows min-max levels

The highest level of Cr (266.78 µg g-1) was found in Pozcu Marina in summer, while the lowest level (77.20 µg g-1) were found at Çamlıbel Marina in spring. Mean levels from Çamlıbel Marina and Karaduvar in spring, and from Deniz Feneri in summer are in the same group and there is a statistically significant difference with the mean levels from Pozcu Marina both in spring and summer seasons. The highest level of Mn was found (305.86 µg g-1) in Pozcu Marina in spring, while the lowest level was found in (32.65 µg g-1) at Çamlıbel Marina in spring. Mean levels from Çamlıbel Marina, Pozcu Marina, Karaduvar in spring, and from Deniz Feneri in summer are in the same group. There is a statistically significant difference in the mean level from Pozcu Marina in summer.

The highest level of As (187.28 µg g-1) was found in Karaduvar in spring. The lowest values (20.44 µg g-1) were found at Çamlıbel Marina in spring. Mean values from Çamlıbel Marina, Pozcu Marina, Deniz Feneri in both spring and summer seasons are in the same group and there is a statistically significant difference with mean values from Karaduvar station in the spring season. The highest level of Se (289.55 µg g-1) Karaduvar in spring, while lowest values (20.26 µg g-1) were found at Deniz Feneri in summer. Mean values from Çamlıbel Marina, Pozcu Marina, Deniz Feneri in both spring and summer seasons are in the same group and there is a statistically significant difference with mean values from Karaduvar station in spring.

The highest level of Sr was found (661.17 µg g-1) in Pozcu Marina in summer, while the lowest level (86.31 µg g-1) were found at Çamlıbel Marina in spring. Mean levels from Çamlıbel Marina and Karaduvar in spring, and from Deniz Feneri in summer are in the same group and there is a statistically significant difference with mean levels from Pozcu Marina in spring and summer seasons. The highest and lowest levels (525.43 - 53.00 µg g-1) were found at Çamlıbel Marina in spring. There is no statistically significant difference in mean level from Çamlıbel Marina in spring. Mean levels from Pozcu Marina and Deniz Feneri in both spring and summer seasons are in the same group and there is a statistically significant difference with mean levels from Karaduvar in spring.

4. DISCUSSION

As a consequence of anthropogenic activities such as mining, agricultural, domestic water disposal, heavy metals contaminate terrestrial and aquatic systems. Also, intense industrial activities produce exhaust gases that include heavy metals (As, Ti, Mo, Mn, Cr, Fe, Zn, Pb, etc.). They are given into the atmosphere, in the form of fine particles heavy metals travel and settle to soil and water. From terrestrial to aquatic environments, these contaminants enter to the food chain and are accumulated reaching levels a thousand times more than the seawater (Castillo 2016). Heavy metals can also enter into environment through natural sources such as; forest fires, volcanic activities, erosion, etc. (Jaishankar et al. 2014). Where there is human population and industry, it can be expected that some level of heavy metal contamination may occur. Therefore biomonitoring of these elements is of great importance for a sustainable environment.

The use of marine organisms as biomonitoring tools is strongly recommended by the Water Framework Directive (2000/60/EC) and the Marine Strategy Framework Directive (2008/56/EC). The green algae from the genus Ulva are the most used macroalgae for the biomonitoring of the levels of trace elements in the marine environment (Chakraborty et al. 2014). While collecting the samples for our study, Ulva genus is abundant and distributed along the coastal line of Mersin. Elemental analyses in our study show that U. rigida accumulates a very high level of heavy metals. These features of U. rigida can present it as an important and valuable indicator in regards to biomonitoring of toxic metals in the coastal area of Mersin (Bonanno and Orlando-Bonaca 2018).

The importance of our analyses and the usage of Ulva genus as a bioindicator in Mersin bay can be better understood when similar studies were reviewed. Malea et al. (2015) also have investigated trace element levels in seawater, sediment, and several macroalgae species including U. rigida. They have made analyses for various heavy metal levels in seawater, sediment, and in four macroalgae species. Despite both studies have the same genus, the results for As levels were notably concentrated in our study. Malea et al. (2015) present mean value of As in U. rigida was 1.449 µg g-1, while in our study U. rigida's lowest value was 23.66 µg g-1. Though it was the lowest value, it was almost 15 times higher than the mean values found in Malea et al. (2015). The mean value of As in seawater was 2.030 µg L-1 in their study. Most notably among our As results, the highest mean value of As was obtained from the Karaduvar station in spring (127.24 µg g-1). The same study also investigated Cr, Mn, Mo, Se, and Sr levels along with several other trace elements, and found mean values respectively Cr: 9.383 µg g-1, Mn: 37.33 µg g-1, Mo: 37.92 µg g-1, Se: 0.281 µg g-1, Sr: 2.707 µg g-1. In recent study, however, lowest mean levels (given with the standard errors for each element) were found respectively; Cr: 95.96 ± 4.93 µg g-1, Mn: 88.61 ± 2.63 µg g-1, Mo: 82.32 ± 0.16µg g-1, Se: 20.83 ± 0.33µg g-1, Sr: 123.36 ± 4.59 µg g-1.

Another study about macroalgae trace element accumulation capacity by Malea and Kevrekidis (2014), also presents trace elements levels on previous studies that investigated Ulva spp., includes six trace elements (As, Cr, Mn, Mo, Se, Sr) in common with the present study. Table 2. shows the range of those trace elements and the results of our study in dry weight.

Table 2. Trace element levels of Ulva spp. from various geographical areas and present study (µg g-1)

Trace element	Levels found in previous studies	The present study (Lowest and highest levels)
As	0.87 - 86	23.66 - 187.28
Cr	0.06 - 84.4	77.20 - 266.78
Mn	0.012 - 1600	32.65 - 305.86
Mo	0 - 58	53.00 - 525.43
Se	< 0.2 - 1.4	20.26 - 289.55
Sr	81 - 700	86.31 - 661.17

There is a clear trace element abundance in all U. rigida samples in Mersin Bay comparing with Malea et al. (2015), also Table 2. clearly shows As, Cr, Mo, Se levels are much higher than they were found in previous studies.

The molybdenum is also an essential element but, in its abundance reduces the intake of copper (Castillo 2016). It should be considered that higher levels of Mo may be already present in the seawater of Mersin Bay. While low selenium status for humans may result in mortality, poor immunity, and cognitive decline, its supplementation, even when daily uptake is adequate, may cause serious human health problems (Rayman 2012). High magnesium content doesn't relate to any kind of hazardous effect on humans, but its deficiency is a serious dietary problem for humans in regards to their physiological functions (Vormann 2003). Potassium also plays a very important physiological role. WHO recommends at least 3510 mg/d K intake for adults (Whelton and He 2014). For their carcinogenic effects, levels of Cr, As, Ti, Al, Mn, and Sr found in the present study should be noted as a potential hazard to human health.

Arsenic is an essential element for life, up to a level (10 µg L-1). However, when it is more than 10 µg L-1, there can be toxicological effects to humans (Fawzy 2008). Arsenic exists in the environment naturally and it can be organic or inorganic form. The toxic effects can occur when the arsenic is in inorganic form (Castillo 2016). Inorganic arsenic is usually an outcome of the use of pesticides which is widely used in agriculture in Mersin. The used irrigation water might be discharged uncontrollably and without any treatment in some areas of Mersin bay. This is very likely to happen for Mersin has a very long coastal area which makes it harder for the authorities to control the irrigation waters. It is also known that arsenic is widely used in industrial activities (processing of glass, textiles, paper, and metal adhesive, etc.) (Garelick et al. 2009). Most of these industrial activities are present in Mersin industrial zones. International Agency for Research on Cancer (IARC) has classified arsenic and arsenic compounds as carcinogenic to humans (Roy and Saha 2002; Castillo 2016)

Strontium-90 is a radioactive contaminant, due to its ~29 year half-time in soil, it is an environmental concern. It occurs in the natural environment solely as the Sr^{2+} ion, and its geochemical behavior is similar to Ca^{2+} (Thorpe et al. 2012). With the Chernobyl and Fukushima accidents, and with disposal of wastewater and sludge related to nuclear activities, large quantities of 90Sr enter the aquatic environments where it can easily be soluble (Martignier et al. 2018). Macroalgae have great strontium accumulation potential, thus they represent great potential for bioremediation as well. In a recent study, Ulva's Sr accumulation capacity in the present study shows similar results with the previous studies. It also shows that Mersin bay may be subject to serious Sr levels. So, its root causes shall be investigated with further research that focuses to monitor the level of this contaminant over the years.

Chromium can be observed in compounds in the environment naturally, but not in elemental form. Contamination of chromium occurs both by electroplating processes and the disposal of Cr-containing waters. Chromium may be transported by water in its soluble form, also precipitated form. Despite Cr is another essential element, Chromium (VI) is carcinogenic to humans (Pathnia 2016). Cr^{3+} is used in the production of leather, steel, and textile while Cr^{6+} is used in electro painting and chemical manufacturing. However, water contamination may be limited because of soil adherence of Cr. When such water contamination occurs, it should be observed whether there is improper disposal of industrial manufacturing equipment (Castillo 2016). EPA set the limit for chromium to be 100 μg L^{-1} in drinking water (EPA, 1999). Glassmaking, cement, and textile manufacturing are some of the sources that can be listed to present in Mersin. The levels found in this study may be linked to industrial activities in Mersin.

High levels of iron and manganese in drinking water may be toxic for organisms. While in lower levels, they play an important role in hemoglobin synthesis. As in the drinking water, their extensive levels accumulated in macroalgae may pose threat through the food chain, and affect neural and muscle systems in humans (Sanjay 2014).

Titanium is known to be inert metal and is used for various implications in the medical industry. Titanium can be found on the earth's crust, also in animals, plants, and natural waters. TiO_2 is used as a food additive and in the cosmetic industry etc. Though in some forms, it can be harmful to human health (Tibau et al. 2019).

Aluminum, when at high levels, is toxic to organisms in aquatic environments. For mammals and birds, aluminum might also be dangerous interfering with their metabolic processes. Thus, its control and monitoring are very important for marine environments (Rosseland et al. 1990).

Considering the hazardous effects of these trace elements in Mersin Bay, our study reveals some seasonality and spatial differences. From our results; Mg levels showed no seasonal difference, but it can be seen that there is a spatial difference between Pozcu Marina and with other three stations. There was no spatial nor seasonal difference regarding Al levels throughout our sampling stations. The most significant spatial difference was found in the accumulation of K levels, which also shows lower levels with a seasonal difference. It can be said that algae might be accumulating less potassium in the summer season. Titanium levels don't show a seasonal difference but due to spatial differences, Pozcu Marina shows the highest levels. Cr and Sr, both showed spatial difference with the accumulated levels in Ulva, particularly high levels were found at Pozcu Marina where it is very close to the city center and a heavily populated area. Mn levels showed no spatial difference, but an increase was found with seasonality. For As, Se, and Mo levels, there was no seasonal difference. Levels of those three elements showed an increase with a spatial difference, in particular at Karaduvar station. It should be also noted due to the possible decay of algae, there was no sampling in the summer season at Karaduvar Station.

5. CONCLUSION

Potentially hazardous trace elements (Al, Ti, Cr, Sr, As, Mn) were found in high levels in all seasons and sampling points in comparison to other studies. The abundance in other elements (Mg, K, Mo, and Se) was also notable in the same sense. The seasonal difference seems to have little effect on the accumulation of trace elements for our study. It should be noted that Mersin bay is subject to high water temperature and salinity. Those factors may play an important role in the decay of algae in the summer season, due to these limitations, sampling in every season was not possible. Though, it can be seen Pozcu Marina and Karaduvar stations are good sampling points in regards to monitor potentially toxic elements in macroalgae tissue. The present study was also conducted comparatively in a short period, longer periods of sampling may result in a better understanding about the accumulation of trace elements. Also in up-to-date studies, levels might be expected to rise due to the recent events (floods and forest fires in Turkey). Those negative chain of events may drive researchers to increase monitoring studies' scope and the time period in the future.

Author contributions

Nahit Soner BÖREKÇİ: Conceptualization, Investigation, Writing- Original draft preparation, Writing Reviewing and Editing.
Mısra BAKAN: Investigation
Büşra PEKSEZER: Investigation
Mehmet Tahir ALP: Writing Reviewing and Editing.
Deniz Ayas: Investigation, Writing- Original draft preparation, Writing-Reviewing and Editing.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence this paper.

Statement of Research and Publication Ethics

The authors declare that this study complies with Research and Publication Ethics.

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