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**RESEARCH ARTICLE** **ARAŞTIRMA MAKALESİ**

# Assessment of polonium-210 bioaccumulation in Mediterranean limpet *Patella caerulea* (Linnaeus, 1758) and sea urchin *Paracentrotus lividus* (Lamarck, 1816) from different coastal areas of Türkiye: Inclusion of a seasonal investigation

# Türkiye'nin farklı kıyı bölgelerinden Çin şapkası, *Patella caerulea* (Linnaeus, 1758) ve deniz kestanesinde *Paracentrotus lividus* (Lamarck, 1816) polonyum-210 biyoakümülasyonunun mevsimsel olarak değerlendirilmesi

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**Abstract:** This study investigated the seasonal variations of polonium-210 ($^{210}Po$) activity concentrations in two marine invertebrate species: Mediterranean limpet *(P. caerulea)* and the sea urchin *(P. lividus)*. Seasonal sample collection was conducted across three Aegean and Sea of Marmara coastal stations from December 2018 to October 2019. The stations included İzmir-Urla, Karaburun, and İstanbul Island-Kınalıada. To assess the size-dependent bioaccumulation of $^{210}Po$, individuals were categorized into size groups. The activity concentrations in both species exhibited seasonal fluctuations, ranging from 4.9 ± 3.4 Bq kg$^{-1}$ dry weight to 28.0 ± 8.4 Bq kg$^{-1}$ dry weight in Mediterranean limpets and 8.7 ± 6.1 Bq kg$^{-1}$ dry weight to 58.0 ± 18.5 Bq kg$^{-1}$ dry weight in sea urchins. The highest $^{210}Po$ activity concentrations were consistently observed in spring across all sampling locations.

**Keywords:** Polonium-210, bioaccumulation, environmental monitoring, marine invertebrates

**Öz:** Bu çalışma, iki deniz omurgasız türünde polonyum-210 ($^{210}Po$) aktivite konsantrasyonlarının mevsimsel değişimlerini incelemiştir: Çin şapkası *(Patella caerulea)* ve deniz kestanesi *(Paracentrotus lividus)*. Aralık 2018'den Ekim 2019'a kadar olan dönemde, Ege ve Marmara Denizi kıyılarında mevsimsel örnek toplama çalışmaları üç farklı istasyonda gerçekleştirildi. Bu istasyonlar İzmir-Urla, Karaburun ve İstanbul Adaları-Kınalıada olarak belirlendi. $^{210}Po$'nun boyuta bağlı birikimini değerlendirmek için numuneler boy gruplarına ayrılmıştır. Her iki türdeki aktivite konsantrasyonları mevsimsel dalgalanmalar göstermiş olup, Çin şapkası örneklerinde 4,9 ± 3,4 Bq kg$^{-1}$ kuru ağırlık ile 28,0 ± 8,4 Bq kg$^{-1}$ kuru ağırlık arasında, deniz kestanelerinde ise 8,7 ± 6,1 Bq kg$^{-1}$ kuru ağırlık ile 58,0 ± 18,5 Bq kg$^{-1}$ kuru ağırlık arasında değişmiştir. Tüm örnekleme istasyonlarında en yüksek $^{210}Po$ aktivite konsantrasyonları sürekli olarak bahar aylarında gözlenmiştir.

**Anahtar kelimeler:** Polonyum-210, biyoakümülasyon, çevresel izleme, deniz omurgasızları

## INTRODUCTION

Marine pollution presents a significant environmental challenge for developed nations, demanding global efforts for control and prevention. Contemporary strategies integrate traditional chemical analysis with biological indicators to assess the impact of pollution on living resources (Beiras et al., 2003). Among these biological tools, embryo-larval bioassays with marine invertebrates, particularly sea urchins and bivalves, are highly developed and extensively employed for global pollution monitoring and evaluation (His et al., 1999).

Sea urchins *(P. lividus)* have emerged as valuable tools for acute bioassays in marine pollution studies due to their sensitivity to pollutants (Dorey et al., 2018; Kobayashi, 1971, 1972, 1990, 1995; Warnau et al., 1996). As bioindicators, Mediterranean limpets *(P. caerulea)* hold particular significance. These widely distributed gastropods, known as Chinese hat shells, exhibit ideal characteristics for pollution monitoring. Their herbivorous diet, sedentary lifestyle on intertidal hard surfaces, and limited mobility simplify the interpretation of pollutant accumulation (Bu-Olayan and Thomas, 2001; Campanella et al., 2001; Cravo et al., 2002; Nakhlé et al., 2006; Pérez et al., 2019; Reguera et al., 2018; Storelli and Marcotrigiano, 2005). Additionally, their documented sensitivity to metal contamination has led to their widespread use in marine pollution monitoring programs (Reguera et al., 2018).

The ever-present threat of environmental pollution in coastal ecosystems necessitates the use of diverse indicator species for effective biomonitoring programs. This approach ensures a wider range of organisms can be utilized to detect a broader spectrum of potential toxic substances and exposure pathways. In this context, limpets (*Patella* spp.) have emerged as promising candidates for biomonitoring, prompting a comprehensive literature review. While not traditionally consumed for human food in Türkiye, limpets play a vital role in the marine food web as a primary food source for fish (Xu and Barker, 1990). They occupy intertidal rocky shores across the Mediterranean and Black Sea basins (Çulha and Bat, 2010).

Among marine contaminants, radioactive isotopes (radionuclides) are of particular concern. This study focuses on $^{210}$Po, a naturally occurring radioisotope with a high alpha energy (5.3 MeV) and a relatively short half-life (138.4 days). Due to its bioaccumulation in marine organisms, $^{210}$Po is the primary radionuclide responsible for internal radiation exposure in both marine life and seafood consumers (Carvalho et al., 2017; Hansen et al., 2022; Kül et al., 2020; Makmur et al., 2020; McDonald et al., 1986; Putri et al., 2022).

Several studies have shed light on the complex interplay between seasonal variations and bioaccumulation processes in marine invertebrates, particularly sea urchins. Lök and Köse (2006) identified peak gonad development in *P. lividus* during February and May, with gonads reaching up to 8.84%-8.97% of their body weight. These findings align with those of Rithu et al. (2022), who observed that fluctuations in $^{137}$Cs activity within sea urchins mirrored dietary changes rather than variations in seawater concentrations. This suggests that seasonal changes in food availability and composition, coinciding with peak gonad development, may significantly influence radionuclide uptake. Reeves et al. (2019) further emphasized this complexity by demonstrating a link between uranium bioaccumulation and both seasonal variations in gonad quantity and protein content. This suggests that internal physiological factors, beyond simply dietary intake, can play a crucial role in radionuclide uptake and accumulation within sea urchins.

By combining these findings, we gain a more comprehensive understanding of the multifaceted nature of bioaccumulation in sea urchins. In general, seasonal variations in both external environmental factors and internal physiological states play a significant role in shaping radionuclide uptake and accumulation patterns (Brown et al., 2024) Further research is needed to fully elucidate the intricate interplay between these factors and their impact on the health and well-being of marine ecosystems.

Sánchez-Marín et al. (2022) explored the potential of limpets as substitutes for mussels in monitoring metal pollution. Their findings suggest that limpet-to-mussel metal concentration ratios can be employed to compare metal concentrations across different regions. This method has been shown to be effective for several metals (As, Cu, Hg, Pb, Cr, Ni, and Zn). However, a notable exception is cadmium (Cd), where no correlation was observed between limpet and mussel concentrations, likely due to differing feeding strategies or detoxification mechanisms for Cd in these organisms.

This study aims to:

1. Determine $^{210}$Po concentrations in the sea urchin *(P. lividus)* and Mediterranean limpet *(P. caerulea)* from three coastal regions of Türkiye: İstanbul Adaları-Kınalıada, İzmir-Urla, and Karaburun.

2. Evaluate the obtained $^{210}$Po data to assess the marine pollution status in these regions.

## MATERIALS AND METHODS

Specimens of the Mediterranean limpet, *P. caerulea*, and the sea urchin, *P. lividus*, were collected from December 2018 to October 2019 across a total of three coastal stations (Figure 1). These stations included two locations in the Aegean Sea: İzmir-Urla (Latitude: 38.370760, Longitude: 26.793942) and Karaburun (Latitude: 38.428651, Longitude: 26.491126), and one station in the Sea of Marmara: İstanbul Island-Kınalıada (Latitude: 40.914077, Longitude: 29.058763). Kınalıada ranks as the fourth-smallest inhabited island amongst the Princes' Islands Archipelago. The Aegean region harbors numerous sprawling industrial centers and major urban populations. Additionally, intensive agricultural practices characterized by substantial fertilizer application are prevalent within the region. Furthermore, several rivers, including the Bakırçay, Gediz, and Menderes, act as conduits, discharging industrial and agricultural pollutants directly into the Aegean Sea.


**Figure 1.** The sampling locations

Field collections of Mediterranean limpet specimens were conducted from December 2018 to October 2019. For each experimental trial, 30–50 adult Mediterranean limpets were collected. Specimens were carefully detached from the substratum using a sterilized knife and transported alive to the laboratory.

Sea urchins were collected seasonally from December 2018 to October 2019. In each season, a sample of greater than fifty adult *P. lividus* individuals was collected from each designated sampling station. SCUBA diving techniques facilitated manual specimen collection at a depth ranging from one to two meters. Upon capture, live animals were promptly transported to the laboratory for processing. Following collection, field samples were transported to the laboratory in labeled plastic bags on ice to maintain sample integrity. Subsequently, the collected individuals were grouped based on their size measurements. Each sample underwent thorough cleaning with distilled water to eliminate any surface impurities. For each sampling season, thirty pooled samples of Mediterranean limpet and sea urchins were homogenized and subsequently divided into different groups based on sizes. Soft tissues from these subsamples were weighed and oven-dried at 70°C, followed by grinding and sieving through a 2 mm mesh. Three 1 g subsamples were prepared for analysis.

Following the addition of a standard polonium-209 tracer, complete sample dissolution was achieved through treatment with HCl and $HNO_3$. Subsequently, polonium underwent spontaneous electrodeposition onto copper discs immersed in 0.5 M HCl. Ascorbic acid's reducing ability is essential for converting ferric ions ($Fe^{3+}$) to ferrous ions ($Fe^{2+}$), preventing thick plating and ensuring efficient deposition with high resolution (Baskaran, 2011, Flynn, 1968).

Quantification of $^{210}$Po relied on the detection of its characteristic 5.30 MeV alpha particle emission. Importantly, an internal tracer consisting of polonium-209 (4.88 MeV alpha emission, $t_{1/2}$ = 109 years) was employed to ensure accuracy. Specific alpha activities were measured by Ortec Octete Plus spectrometry system. $^{209}$Po (4.88 MeV alpha emission, $t_{1/2}$ = 103 years) was used as the internal tracer (Standard Reference Material 4326). The chemical yields using the $^{209}$Po tracer ranged between 70 and 90%. The detection limit of the alpha spectrometry system is 0.0003 Bq.

The results were analyzed with one-way ANOVA test via IBM SPSS 23 statistical program and the Microsoft Excel packages. The differences were evaluated at the 5% significance level ($P<0.05$).

## RESULTS

The $^{210}$Po activity concentrations in the Mediterranean limpet *(P. caerulea)* and sea urchin *(P. lividus)* species samples are given in Figure 2, Figure 3. The average dry weight to wet weight ratios for the Mediterranean limpet *(P. caerulea)* and sea urchin *(P. caerulea)* species are 0.25 and 0.23, respectively.

The $^{210}$Po activity concentrations in the Mediterranean limpet *(P. caerulea)* species samples varied between 4.9±3.4 Bq kg$^{-1}$ dry weight – 28.0±8.9 Bq kg$^{-1}$ dry weight.

For the Mediterranean limpet samples, while preliminary observations suggested highest values in spring and lowest in summer for all stations, a more detailed examination incorporating error bars indicates a different pattern. Specifically, the lowest concentrations were detected in both autumn and summer, whereas the highest values occurred during winter and spring. Furthermore, a notable exception to this trend was observed for Mediterranean limpets in the 4 cm-<5 cm size category at Kınalıada station, which exhibited elevated $^{210}$Po levels in autumn. A one-way ANOVA was employed to determine if significant differences existed in specific $^{210}$Po activity concentrations among stations, seasons, and size categories. Statistical analysis indicated no significant correlation ($P > 0.050$) between specific $^{210}$Po activity concentrations and station or size. However, there was a significant correlation ($p < 0.000$) between $^{210}$Po activity concentrations and season. Descriptive statistics of $^{210}$Pb activity concentrations in *P. caerulea* individuals are given in Table 1. To the best of our current knowledge, no prior studies have reported $^{210}$Po activity concentrations in Mediterranean limpet samples.

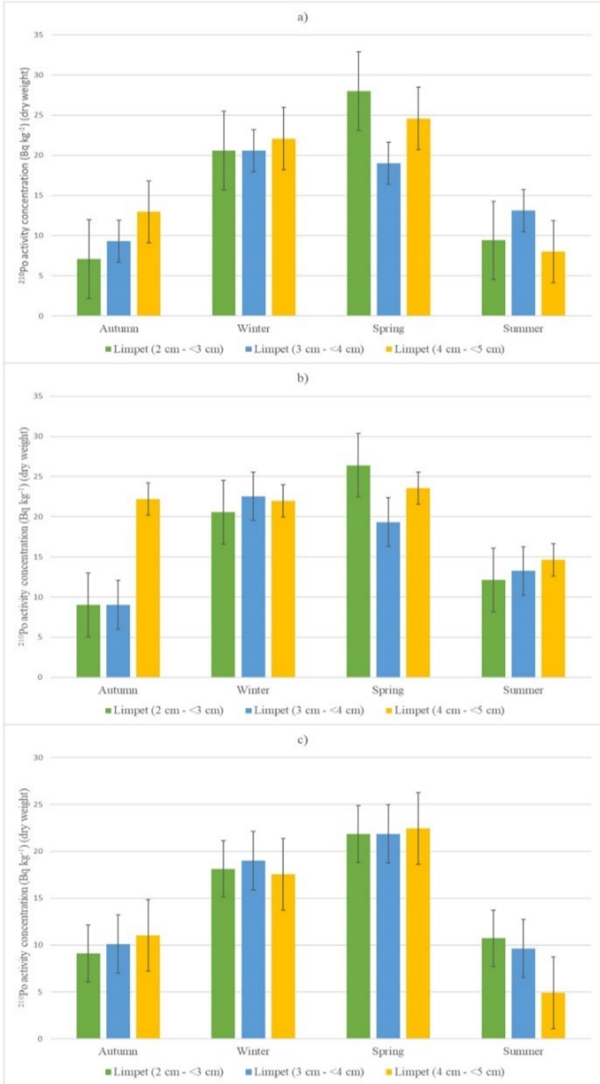


**Figure 2.** a) Seasonal variations of the $^{210}$Po activity concentrations in the Mediterranean limpet *(P. caerulea)* samples Urla station, b) Seasonal variations of the $^{210}$Po activity concentrations in the Mediterranean limpet *(P. caerulea)* samples Kınalıada station, c) Seasonal variations of the $^{210}$Po activity concentrations in the Mediterranean limpet *(P. caerulea)* samples Karaburun station

The $^{210}$Po activity concentrations in the sea urchin *(P. lividus)* species samples varied between 8.7±6.1 Bq kg$^{-1}$ dry weight – 58.0±18.6 Bq kg$^{-1}$ dry weight.

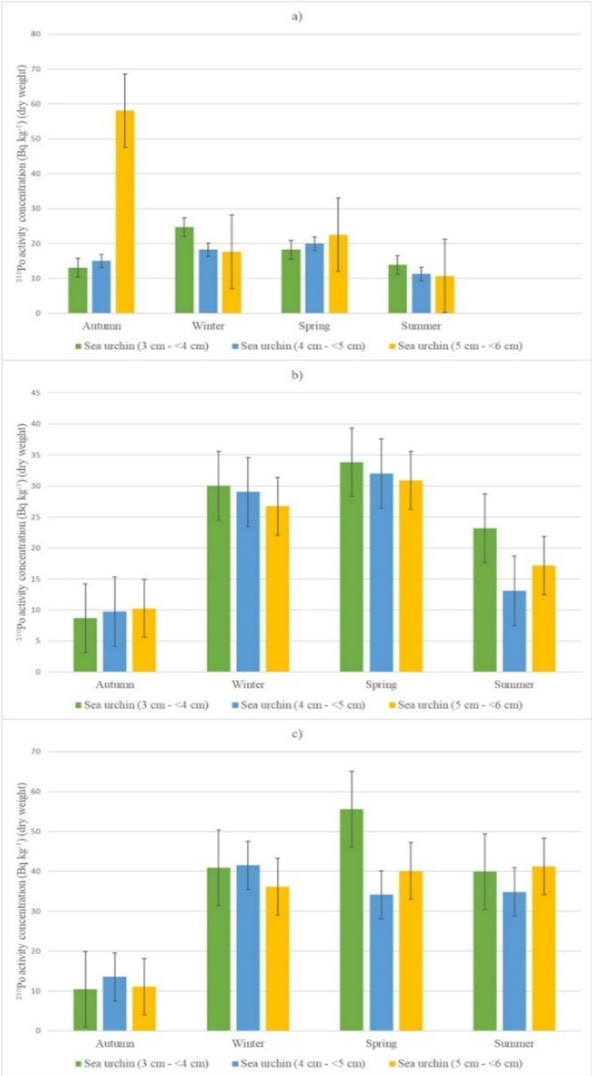


**Figure 3.** a) Seasonal variations of the $^{210}$Po activity concentrations in the Sea urchin *(P. lividus)* samples Urla station, b) Seasonal variations of the $^{210}$Po activity concentrations in the Sea urchin *(P. lividus)* samples Kınalıada station, c) Seasonal variations of the $^{210}$Po activity concentrations in the Sea urchin *(P. lividus)* samples Karaburun station

**Table 1.** Descriptive statistics of *P. caerulea* $^{210}$Pb activity concentrations and stations, seasons, sample sizes

| Station | Mean | ±SE | ±SD | Minimum | Maximum | P |
|---|---|---|---|---|---|---|
| Urla | 16.2192 | 2.04853 | 7.0963 | 7.1 | 28 | |
| Kınalıada | 17.8825 | 1.72596 | 5.9789 | 9.01 | 26.40 | 0.482 |
| Karaburun | 14.6950 | 1.75007 | 6.0624 | 4.91 | 22.45 | |
| **Season** | **Mean** | **±SE** | **±SD** | **Minimum** | **Maximum** | **P** |
| Autumn | 11.0899 | 1.48950 | 4.4685 | 7.1 | 22.21 | |
| Winter | 20.3289 | 0.59014 | 1.7704 | 17.56 | 22.54 | 0.000* |
| Spring | 23.0010 | 0.99823 | 2.9947 | 19 | 28 | |
| Summer | 10.6424 | 1.00914 | 3.0274 | 4.91 | 14.64 | |
| **Sample Size** | **Mean** | **±SE** | **±SD** | **Minimum** | **Maximum** | **P** |
| 2 cm-<3 cm | 16.0808 | 2.12171 | 7.3498 | 7.1 | 28 | |
| 3 cm-<4 cm | 15.5584 | 1.53148 | 5.3052 | 9.01 | 22.54 | 0.829 |
| 4 cm-<5 cm | 17.1574 | 1.93192 | 6.6924 | 4.91 | 24.58 | |

*There are statistically differences between the $^{210}$Pb activity concentrations and stations, seasons sample sizes; Mean, average $^{210}$Pb activity concentration, SE, Standard Error; SD, Standard Deviation; P, Significance

The highest $^{210}$Po activity concentrations in sea urchin (*P. lividus*) samples were observed in individuals measuring 5-6 cm in the autumn at Urla station. While lower concentrations were found in other size categories, the data indicate a clear peak in this particular group during this season. Conversely, the lowest $^{210}$Po activity concentrations were recorded in autumn at Kınalıada station. No significant correlation ($p > 0.05$) was observed between specific $^{210}$Po activity concentrations and seasonal variations or sample sizes within the sea urchin population. A significant difference ($p<0.029$) in $^{210}$Po concentrations was observed among stations for sea urchin samples. Descriptive statistics of $^{210}$Pb activity concentrations in *P. lividus* individuals are given in Table 2. In a study conducted by Hurtado-Bermúdez et al. (2019) on Sea Urchin samples collected in Spain, the authors reported $^{210}$Po activity concentrations ranging from 38 to 61.5 Bq kg$^{-1}$ dry weight.

**Table 2.** Descriptive statistics of *P. lividus* $^{210}$Pb activity concentrations and stations, seasons, sample sizes

| Station | Mean | ±SE | ±SD | Minimum | Maximum | P |
|---|---|---|---|---|---|---|
| Urla | 20.2742 | 3.64895 | 12.6404 | 10.72 | 58.01 | |
| Kınalıada | 22.0579 | 2.78508 | 9.6478 | 8.69 | 33.81 | 0.029* |
| Karaburun | 33.2957 | 4.07404 | 14.1129 | 10.44 | 55.58 | |
| **Season** | **Mean** | **±SE** | **±SD** | **Minimum** | **Maximum** | **P** |
| Autumn | 16.6537 | 5.21246 | 15.6404 | 8.69 | 58.01 | |
| Winter | 29.4444 | 2.92950 | 8.7885 | 17.62 | 41.55 | 0.057 |
| Spring | 31.9187 | 3.82481 | 11.4744 | 18.25 | 55.58 | |
| Summer | 22.8202 | 4.18922 | 12.5677 | 10.72 | 41.23 | |
| **Sample Size** | **Mean** | **±SE** | **±SD** | **Minimum** | **Maximum** | **P** |
| 3 cm-<4 cm | 26.0396 | 4.16800 | 14.4383 | 8.69 | 55.58 | |
| 4 cm-<5 cm | 22.7065 | 3.16645 | 10.9622 | 9.73 | 41.55 | 0.729 |
| 5 cm-<6 cm | 26.8817 | 4.29628 | 14.8828 | 10.27 | 58.01 | |

*There are statistically differences between the $^{210}$Pb activity concentrations and stations, seasons sample sizes; Mean, average $^{210}$Pb activity concentration, SE, Standard Error; SD, Standard Deviation; P, Significance

## DISCUSSION

The study investigated the bioaccumulation of Polonium-210 ($^{210}$Po) in two marine invertebrate species: the Mediterranean limpet (*P. caerulea*) and the sea urchin (*P. lividus*). This could be attributed to seasonal changes in phytoplankton abundance, a primary food source for sea urchins known to accumulate these radionuclides. It is important to acknowledge, however, that limitations in our understanding of uptake pathways and bioaccumulation capacities for PAHs prevent a definitive attribution of the observed peak spring concentration solely to biological and physiological processes. (Bartolomé et al., 2011) reported a similar seasonal pattern in PAH concentration profiles for other sentinel organisms like mussels and oysters, suggesting broader environmental factors may be at play.

The review process, encompassing 88 studies identified on the Web of Science platform, further strengthens the case for limpet suitability in biomonitoring programs. Numerous field studies have documented the capacity of limpets to accumulate both metals and hydrocarbons. In many cases, a clear link exists between the level of a pollutant in the surrounding environment and the corresponding body content of the pollutant within the limpet's soft tissues. Additionally, research has revealed various physiological responses in limpets exposed to pollutants. These responses include DNA damage induction, metallothionein induction, oxidative stress, reduced Neutral Red retention, and variations in heart rate. While some Patella species exhibit varying responses to disturbances (e.g., oil spills, wastewater discharge), the overall trend suggests their sensitivity is comparable to, or even surpasses, that of mussels. This, coupled with their demonstrated ability to accumulate pollutants, makes limpets strong candidates for inclusion as sentinel organisms in regional monitoring plans (Reguera et al., 2018).

There was an observed seasonal variation in the activity concentrations of $^{210}$Po for both Mediterranean limpet and sea urchin species. Higher activity concentrations were consistently detected in samples collected during the springtime. However, these seasonal variations were not statistically significant. This likely reflects seasonal changes in phytoplankton abundance, a primary food source known to accumulate these radionuclides. Existing research suggests that internal factors beyond diet, such as gonad development cycles, may influence radionuclide uptake in sea urchins. A comprehensive understanding of bioaccumulation in marine organisms like sea urchins is critical for assessing the health of marine ecosystems. Seasonal variations in both environmental factors (e.g., food availability) and internal physiological states significantly impact radionuclide uptake patterns. Further research is needed to fully elucidate the complex interplay between environmental factors, internal physiology, and their impact on radionuclide bioaccumulation in marine organisms. Investigating the impact of anthropogenic activities, such as pollution from disused mines, on marine life using sensitive bioassays can be valuable for environmental monitoring and mitigation efforts (Jewel et al., 2002; Santhanabharathi et al., 2023; Stewart et al., 2008; Thiessen at al., 1999). While not traditionally consumed in Türkiye, limpets (*Patella* spp.) serve as a vital food source for fish. Their declining populations due to pollution and habitat loss necessitate further investigation to ensure their long-term sustainability.

This study documented elevated $^{210}$Po concentrations within both *Paracentrotus lividus* and *Patella caerulea* during the spring and winter seasons. It is noteworthy that the observed elevation in $^{210}$Po levels during spring did not achieve statistical significance. Seasonal fluctuations in phytoplankton abundance, a primary food source for these sea urchins and known concentrators of $^{210}$Po, may be a contributing factor. The timing of sea urchin spawning is hypothesized to be strategically linked to peak phytoplankton blooms, ensuring a readily available food source for their developing offspring. This coincides with a seasonal cycle where spring ushers in phytoplankton blooms, followed by sea urchin reproduction in spring/summer to capitalize on this abundance (Padilla-Gamiño et al., 2022; Peck et al., 2005). Phytoplankton declines in summer/fall as nutrients are depleted, and winter finds sea urchins utilizing alternative food sources or entering a period of reduced activity (Khaili et al., 2024). However, limitations in our current understanding of PAH uptake pathways and bioaccumulation capacities in these species hinder a definitive attribution of the observed springtime peak solely to biological processes (Bartolomé et al., 2011). Bartolomé et al. (2011) reported a similar seasonal trend in PAH concentrations within other sentinel organisms, such as mussels and oysters, suggesting the influence of broader environmental drivers.

A review conducted by (Reguera et al., 2018) analyzing 88 studies identified on the Web of Science platform strengthens the argument for the suitability of limpets in biomonitoring programs. Extensive field research has documented the ability of limpets to bioaccumulate both metals and hydrocarbons (Pérez et al., 2019; Viñas et al., 2018). This bioaccumulation often demonstrates a positive correlation between the level of a pollutant in the surrounding environment and the corresponding concentration found within the limpet's soft tissues (Nuñez et al., 2012). Additionally, research has revealed various physiological responses in limpets exposed to pollutants, including DNA damage induction, metallothionein induction, oxidative stress, reduced Neutral Red retention, and variations in heart rate (Prusina et al., 2014; Sun et al., 2023; Virgin and Schiel, 2023). While some Patella species may exhibit differing sensitivities to specific disturbances (e.g., oil spills, wastewater discharge), the overall trend suggests their sensitivity to pollutants is comparable to, or potentially exceeds, that of mussels (Viñas et al., 2018). This, combined with their documented bioaccumulation capabilities, positions limpets as strong candidates for inclusion as sentinel organisms in regional monitoring plans.

## CONCLUSIONS

This study highlights the importance of multi-faceted research approaches in understanding and managing marine ecosystems. By combining investigations of dietary influences on bioaccumulation with monitoring of pollutant impacts, we can ensure the well-being of these ecologically and economically valuable marine organisms.

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## AUTHORSHIP CONTRIBUTIONS

Duygu Arslantürk: Field work, lab analysis, formal analysis, methodology. Aysun Uğur Görgün: Conceptualization, formal analysis, methodology, funding acquisition, investigation, project administration, resources, supervision, writing - review & editing, Işık Filizok: Conceptualization, investigation, validation, writing - review & editing.

## CONFLICT OF INTEREST STATEMENT

The authors declare they have no conflicts of interest.

## ETHICS APPROVAL

Ethics Committee approval certificate is not required for materials used in this study. For this reason, Ethics Committee Certificate was not obtained in this study.

## DATA AVAILABILITY

All relevant data is in the article.

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