Study of Heavy Metal Pollution in Marine Brown Algae in the Sinop Coasts (Turkey)

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Abstract
This study aimed to determine the seasonal variation of heavy metal pollution in Marine brown algae (Phaeophyta) in the Sinop coasts of Turkey. Samples were collected from two stations in the Sinop coasts in December 2016, March 2017, June 2017 and September 2017. After that, Mn, Cd, Zn, Cu, Fe, Ni and Pb concentrations of these samples were determined. After performing the necessary laboratory analysis and statistical analysis, the results showed that there were no significant differences between the two study areas in Mn, Pb, Zn where the value of the observed level of significance was ($p>0.05$). However, there were significant differences between the two study areas in Ni, Fe, Cu, Cd where the value of the observed level of significance was ($p<0.05$). The results of the heavy metal analysis revealed that there were significant statistical differences among all seasons where the value of the observed level of significance was ($p<0.05$). It can be said that heavy metal pollution in the coasts of Sinop has not reached a dangerous level yet. Finally, it is highly recommended that similar pollution studies should be carried out at regular intervals and reported routinely to the competent authorities.

Keywords: *seasonal, accumulation, Sinop, Marine brown algae*

1. Introduction

The pollution of marine life caused by heavy metal is of prime concern globally. The Dead Sea is characterized as being semi-enclosed. Toxickinetics of heavy metals in the marine environment have long been the subject matter of research considering that its anticipated impact exceeds the plant and animal life to include humans via the food chain (Boran and Altınok, 2010). Chemical pollution largely contributed to the collapse of the Black Sea ecosystems. The profound size of the hydrographical basin with its hydrobiological properties shapes the distinct ecosystem of the Black Sea which, in turn, becomes susceptible to the aforementioned threats (Jitar et al., 2013). The brown algae including Phaeophyceae - are a vast and distinct class of golden-brown algae of various sizes which can be in a microscopic filamentous form, or it can grow to large, complex seaweeds. Phaeophytes consist of plastids with a girdle lamella, thylakoids in accumulates of three, and chloroplast ER. All bear a disproportionate dynamic phase (unequal flagella) and chlorophylls a, c1, and c2, β-carotene, diatoxanthin, and fucoxanthin as photosynthetic pigments (Wehr, 2015). Algae have great potentials in numerous areas of industry and elsewhere. Kumar, Patel, Viyol and Bhoi (2009)explained that seaweeds have been utilized as a valuable source of nutrition for humans and animals, a rich soil fertilizer, salt extraction, medicament, cosmetics, a source of energy, and colloid production; such as agar, furcellaran, alginates, carrageenan, n etc (Bird and Benson,1987). Economically, natural seaweeds are considered as a vital resource mainly in the Indian as well as the Pacific regions in which they are harvested and chiefly exploited as human food sustenance and as nutritional supplements. On the other hand, the aggregation and usage of seaweeds within the European continent is not as pervasive as it is in the Indian and Pacific regions. However, extracting iodine and soda from fucales has been considered a common practice since the 17th century, and seaweeds made a good fodder for livestock which graze on the Northern coastal areas in Europe such as Scotland, Norway, Iceland, etc. The recognition of the importance of seaweeds occurred, for the most part, subsequent to the World War II (Sfriso, Pavoni, Marcomini & Orio 1992), (Schramm, 1991; Schramm, 1996), (Briand, 1991), (Ménesguen and Piriou, 1995), (Morand and Briand, 1996), (Sfriso and Marcomini, 1996). According to Giusti (2001), scrutinizing brown seaweed - contaminated with heavy metal which is located in the UK's coastline in the area between the Wear river and the Tees river - shows that algae from Whitburn, Roker, Easington, and Horden, have a fairly high concentrations of Zn, Cu, Cr, while the burden of Ni and Pb is high only at the latter three locations. Furthermore, Cd and Ag are high at Easington and Horden. These Cd and Ag levels are predominantly akin to those found in polluted estuarine and coasts around the UK. Algae at Holy Island and Bran Sands appear to be the least affected with heavy metals. Evidence shows seasonal fluctuation of heavy metal concentration in macroalgae (Bryan and Hummerstone, 1973), (Fuge and James, 1973, 1974), (Young, 1975), mainly for Mn, Fe, Zn, Cu, Cd, Co, and Al. The variation is evident as there is an increase of concentration in the winter and the beginning of spring, while the summer and autumn seasons show a decrease or minimal concentration. Humphries (1996) stated that in some situations, a reformulation of coal waste into coastal ridges, as a result of erosions, create a layer ofclay minerals and iron oxide over sand particles (Giusti, 2001).

2. Materials and Methods

2.1. Study area.

The Black Sea is the world's largest interior body of water that is globally recognized for its pollution. Approximately, a third of the European continental land infiltrates into it and the Black Sea environment has experienced deterioration from the refuse from around 17 countries (Bat, Gökkurt, Sezgin, Üstün & Sahin, 2009). Sinop province is located at the upmost northern point of the Turkish Black Sea on Boztepe peninsula (Bat, Sezgin, Şahin, Özdemir & Ürkmez 2013). Study samples were collected from two sites in Sinop, and they were taken 4 times in December 2016, March 2017, June 2017 and September 2017.

Fig. 1.Site 1

Fig.2. Site2

2.2. Sampling methods.

The brown algae samples were collected by hand from the aforementioned coastal sites. After that, they were washed with ambient water to remove clay sands, dusts, associated algae, sediments and debris. The cleaned algae were then placed separately in polythene plates. Finally, the seaweeds were shade dried for 10 days in a clean environment to prevent it from defilement (Kannan, 2014).

Fig .3.Image of brown algae samples

2. 3. Sample analysis.

Heavy metal analyses were performed in Kastamonu University Central Research Laboratory. For brown algae samples, 0.5g of each sample was taken and HNO_3 and H_2O_2 were added. The samples were then dried under a pressure of 200°C and 45 bar for 15 minutes and then cooled to room temperature. After cooling, the samples were added to ultra-pure water and the readings were performed in ICP-OES (SpectroBlue). The ICP-OES device used performs three readings for each heavy metal and yields in ppb. There is a dilution factor 200 for all samples. Therefore, the results obtained were multiplied by 200 and all results were divided by 1000 and converted to ppm.

3. Statistical Analysis

For statistical analysis, Mann Whitney test and Kruskal Wallis test were applied to the data following analysis of variance (ANOVA). All statistical analyses were performed with SPSS version for Windows between heavy metals,

Table 1. Results

season	site	cu (ppm)	cd (ppm)	pb (ppm)	ni (ppm)	mn (ppm)	fe (ppm)	zn (ppm)
autumn	1	27.9766	1.1946	6.6986	15.763	78.6662	1052.82	20.7676
	1	27.9266	1.2116	6.8924	15.8924	78.746	1054.944	20.8474
	1	27.8542	1.1928	6.7522	16.01	78.6878	1057.202	20.9498
winter	1	13.7586	0.4272	2.0768	4.3862	11.096	103.2872	10.4136
	1	14.033	0.3868	1.9562	4.3004	10.9956	101.0392	10.5054
	1	14.0382	0.3922	1.9966	4.3314	10.9178	101.0218	10.4812
spring	1	16.2678	0.458	2.7728	3.8022	15.3906	136.107	13.5494
	1	16.1176	0.4684	2.897	3.7984	15.3884	136.8992	13.5604
	1	16.1234	0.4608	2.9316	3.7794	15.4548	138.7586	13.5804
summer	1	22.794	0.492	3.0614	7.5412	12.4304	153.7226	12.6236
	1	23.0412	0.4954	3.0834	7.3794	12.3578	153.0336	12.6188
	1	22.5366	0.4548	3.1266	7.4794	12.2346	151.3176	12.5404
autumn	2	16.6142	0.6168	3.0706	5.366	112.8364	480.332	20.0354
	2	16.476	0.606	3.2576	5.2934	113.8878	484.386	20.1404
	2	16.4726	0.6392	3.1834	5.1812	112.2836	479.254	20.0876
winter	2	8.0764	0.439	2.5064	5.2728	10.6	96.891	12.9382
	2	8.114	0.439	2.5966	5.1794	10.0338	94.6918	12.9968
	2	8.2656	0.4228	2.33	5.205	10.0556	94.3762	12.9624
spring	2	8.5328	0.3386	2.3612	2.0048	11.3184	66.2756	9.2442
	2	8.1714	0.3118	2.3908	1.8474	11.329	66.3738	9.246
	2	8.136	0.3134	2.2926	1.8286	11.3048	66.3708	9.1986
summer	2	10.143	0.369	2.5262	2.2768	11.5672	78.27	12.1748
	2	10.1054	0.3568	2.389	2.2956	11.4896	78.215	12.1126
	2	10.0782	0.3576	2.3914	2.3216	11.5698	79.1516	12.124

Table 2. Comparison between Cu (ppm) in the two study areas

site	samples	Mean	Std. Deviation	Std. Error Mean
1	12	20.2057	5.76243	1.66347
2	12	10.7655	3.56528	1.02921

The results showed that there were significant differences between the two regions where the value of the observed level of significance was (0.001) which is less than 0.05.

Fig. 4. Averages Cu (ppm) with the standard error in the two study areas

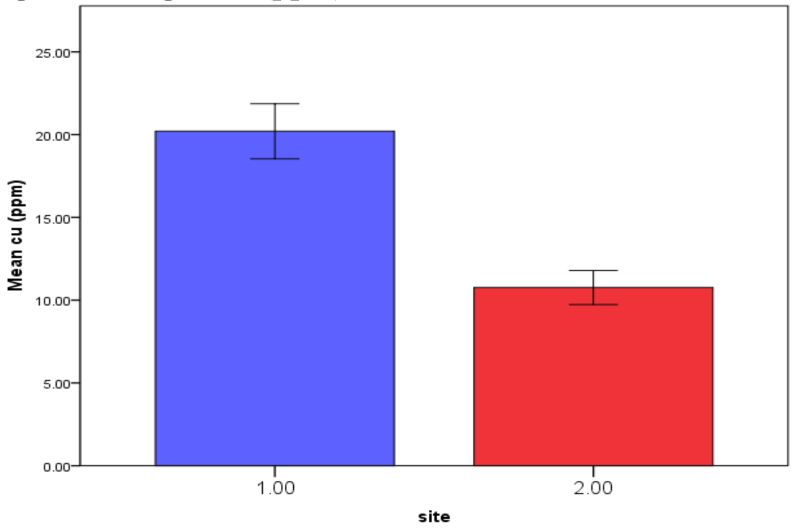


Table 3. Comparison between Cd(ppm) in the two study areas

site	Samples	Mean	Std. Deviation	Std. Error Mean
1	12	.6362	.34143	.09856
2	12	.4342	.12056	.03480

The results showed that there were significant differences between the two regions where the value of the observed level of significance was (0.033) which is less than 0.05.

Fig. 5. Averages with the standard error of Cd (ppm) in the two study areas

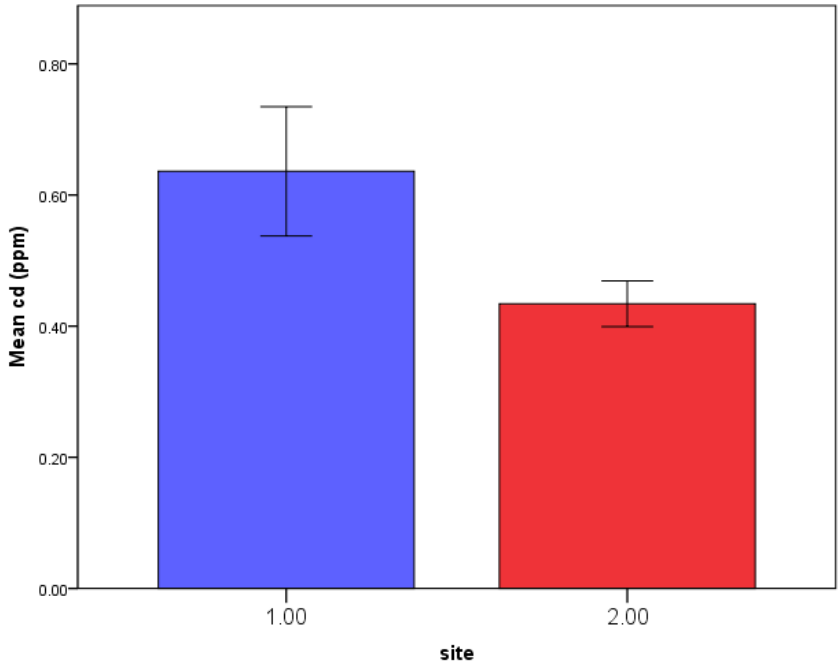


Table 4. Comparison between Pb(ppm) in the two study areas

site	samples	Mean	Std. Deviation	Std. Error Mean
1	12	3.6871	1.91373	.55245
2	12	2.6080	.35213	.10165

The results showed that there were no significant differences between the two regions where the value of the observed level of significance was (0.266) which is greater than 0.05.

Fig. 6. Averages with the standard error of Pb(ppm) in the two study areas

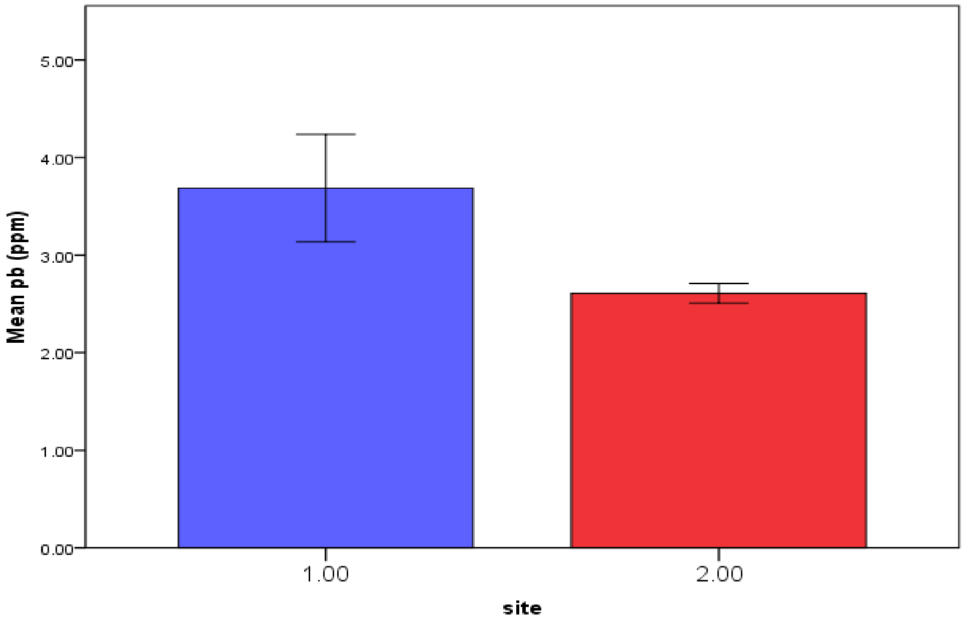


Table 5. Comparison between the Ni (ppm) in the two study areas

Site	samples	Mean	Std. Deviation	Std. Error Mean
1	12	7.8720	5.05135	1.45820
2	12	3.6727	1.65509	.47778

The results showed that there were significant differences between the two regions, where the value of the observed level of significance was (0.039) which is less than 0.05.

Fig. 7. Averages with the standard error of Ni (ppm)in the two study areas

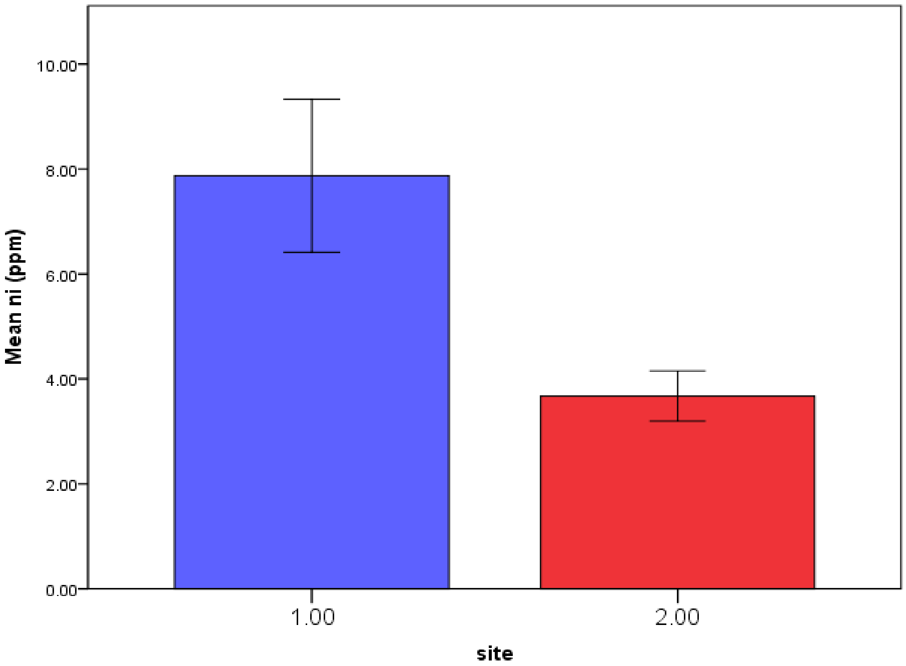


Table 6. Comparison between the Mn (ppm) in the two study areas

Site	samples	Mean	Std. Deviation	Std. Error Mean
1	12	29.3638	29.79768	8.60185
2	12	36.5230	46.12336	13.31467

The results showed that there were no significant differences between the two regions where the value of the observed level of significance was (0.319) which is greater than 0.05.

Fig.8. Averages with the standard error Mn (ppm) in the two study areas

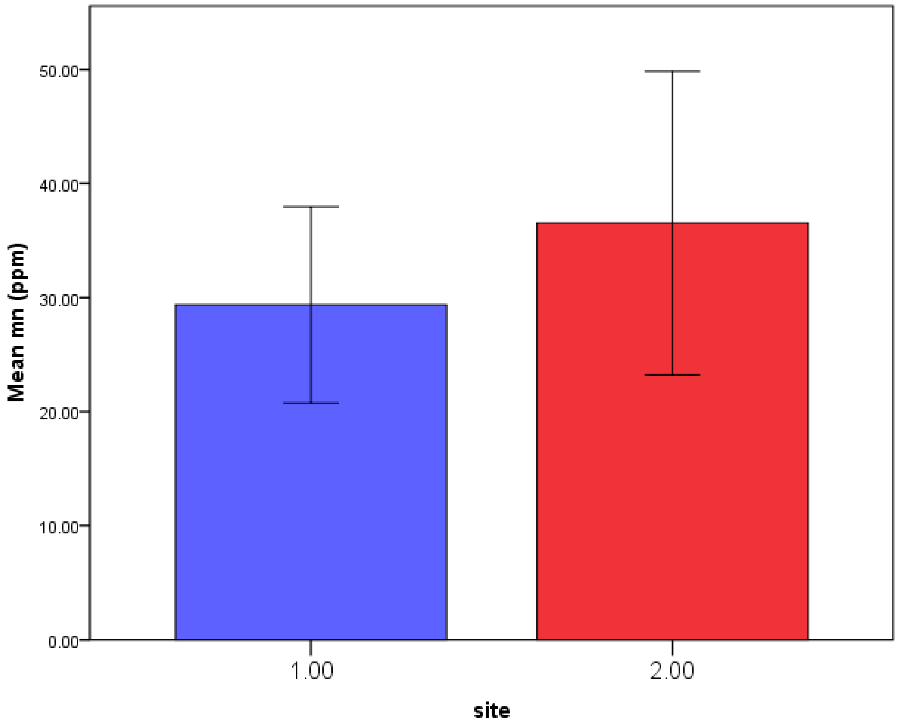


Table 7. Comparison between the Fe (ppm) in the two study areas

Site	samples	Mean	Std. Deviation	Std. Error Mean
1	12	361.6794	418.52761	120.81851
2	12	180.3823	181.79727	52.48035

The results showed that there were significant differences between the two regions, where the value of the observed level of significance was (0.008) which is less than 0.05.

Fig.9. Averages with the standard error of Fe (ppm) in the two study areas

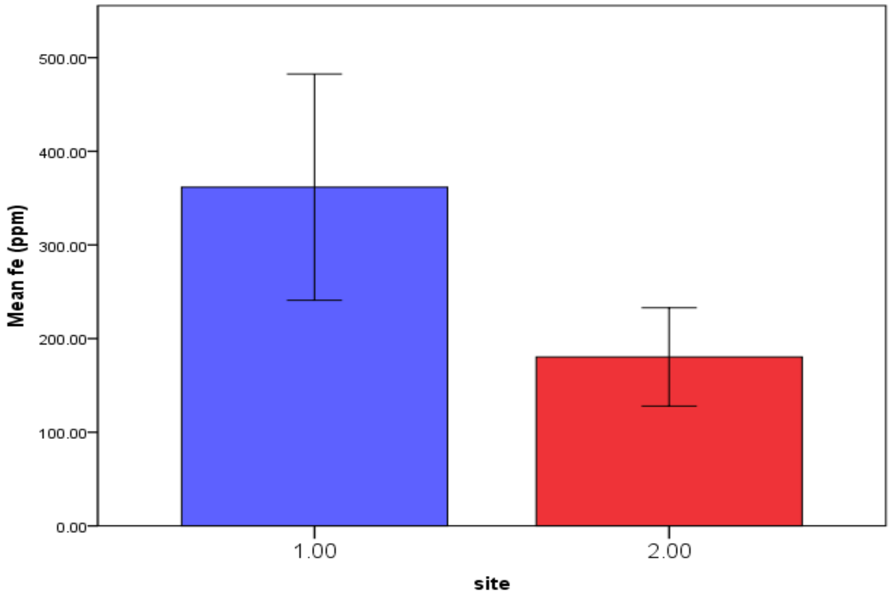


Table 8. Comparison between Zn (ppm) in the two study areas

Site	samples	Mean	Std. Deviation	Std. Error Mean
1	12	14.3698	4.08219	1.17843
2	12	13.6051	4.16929	1.20357

The results showed that there were no significant differences between the two regions where the value of the observed level of significance was (0.319) which is greater than 0.05.

Fig. 10. Averages with the standard error of Zn (ppm) in the two study areas

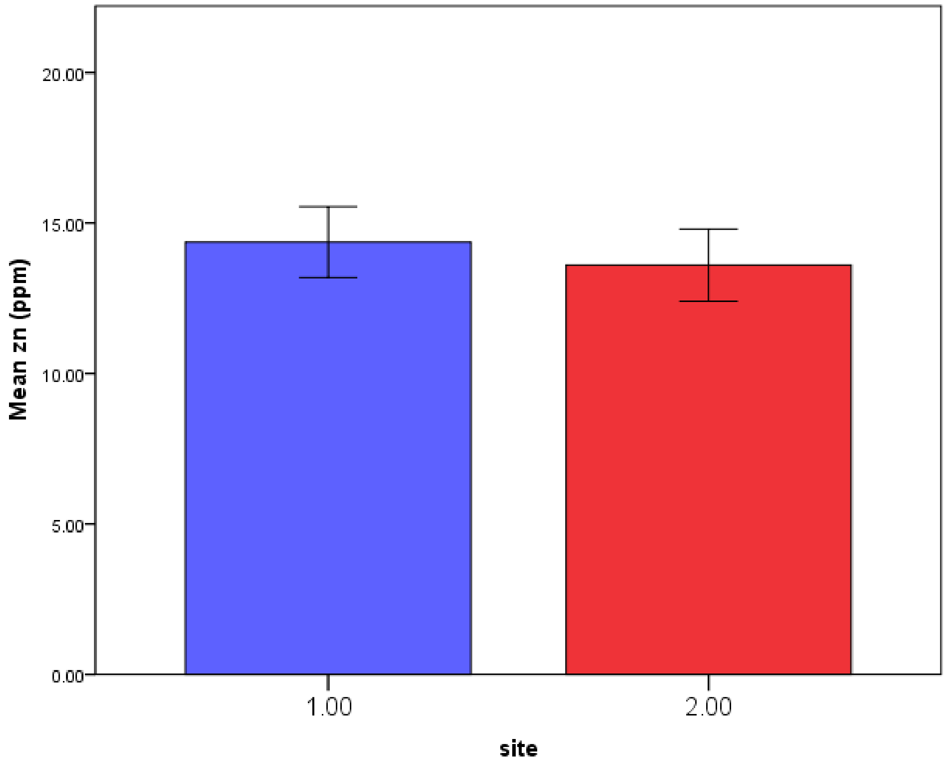


Table 9. Comparison between the of Cu (ppm) in the seasons

seasons	samples	Mean	Std. Deviation	Std. Error Mean
autumn	6	22.2200	6.24338	2.54885
winter	6	11.0476	3.17425	1.29588
spring	6	12.2248	4.32384	1.76520
summer	6	16.4497	6.94793	2.83648

The results showed that there were significant differences between the seasons of the year where the value of the observed level of significance was (0.007) which is less than 0.05.

Fig. 11. Averages with the standard error of Cu (ppm) in the seasons

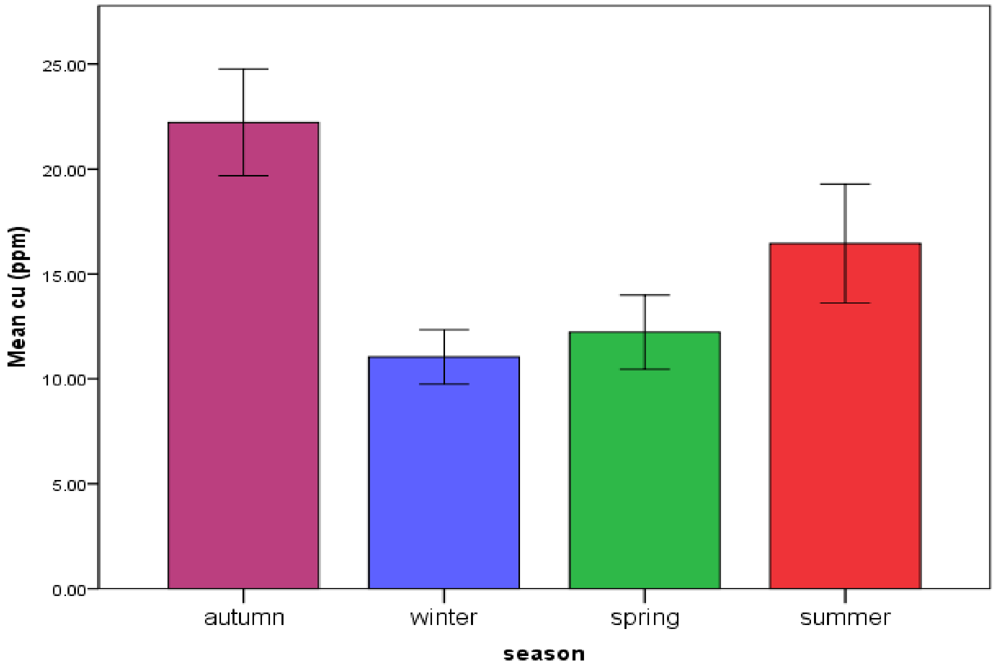


Table 10. Comparison between Cd (ppm) in the seasons

seasons	samples	Mean	Std. Deviation	Std. Error Mean
autumn	6	.9102	.31738	.12957
winter	6	.4178	.02293	.00936
spring	6	.3918	.07796	.03183
summer	6	.4209	.06718	.02743

The results showed that there were significant differences between seasons of the year where the value of the observed level of significance was P-value equal to (0.004) which is less than 0.05.

Fig.12. Averages with the standard error of Cd (ppm) in the seasons

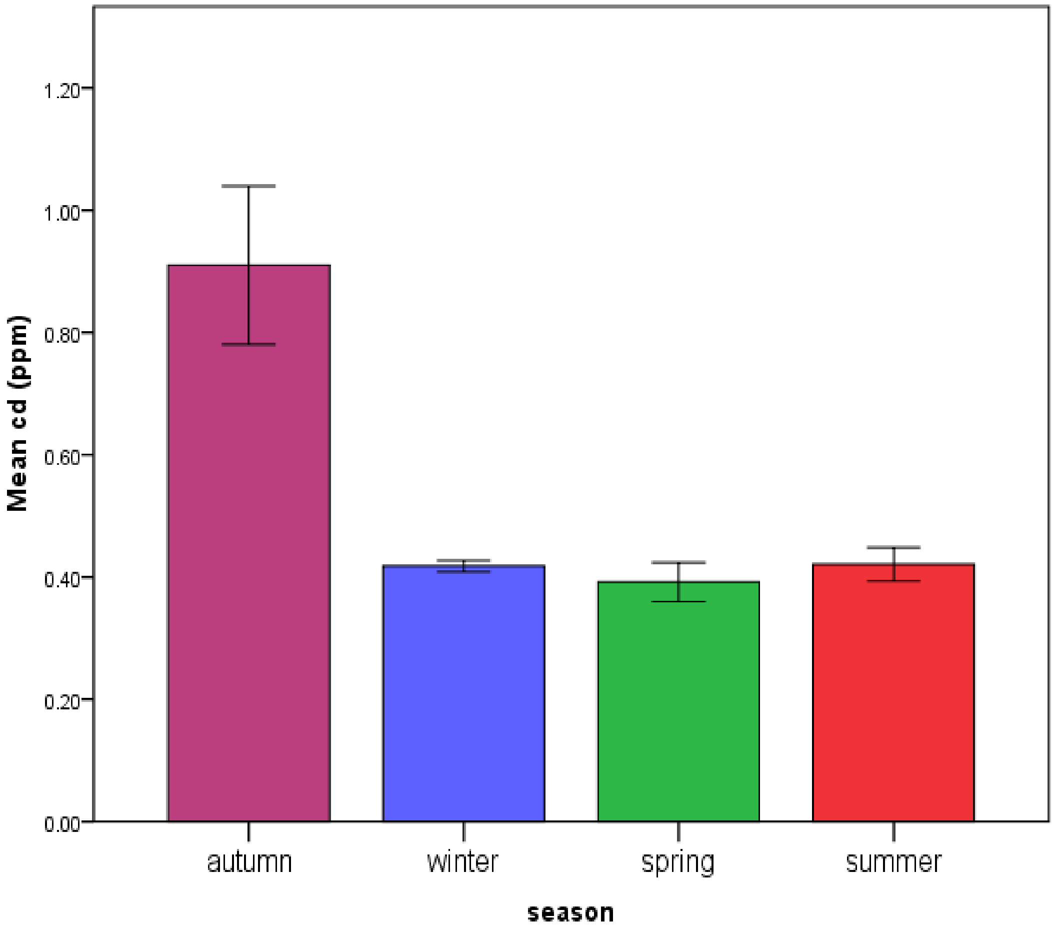


Table 11. Comparison between the of pb (ppm) in the seasons

seasons	samples	Mean	Std. Deviation	Std. Error Mean
autumn	6	4.9758	1.97948	.80812
winter	6	2.2438	.27297	.11144
spring	6	2.6077	.29085	.11874
summer	6	2.7630	.36275	.14809

The results showed that there were significant differences between seasons of the year where the value of the observed level of significance was (0.001) which is less than 0.05.

Fig. 13. Averages with the standard error of pb (ppm) in the seasons

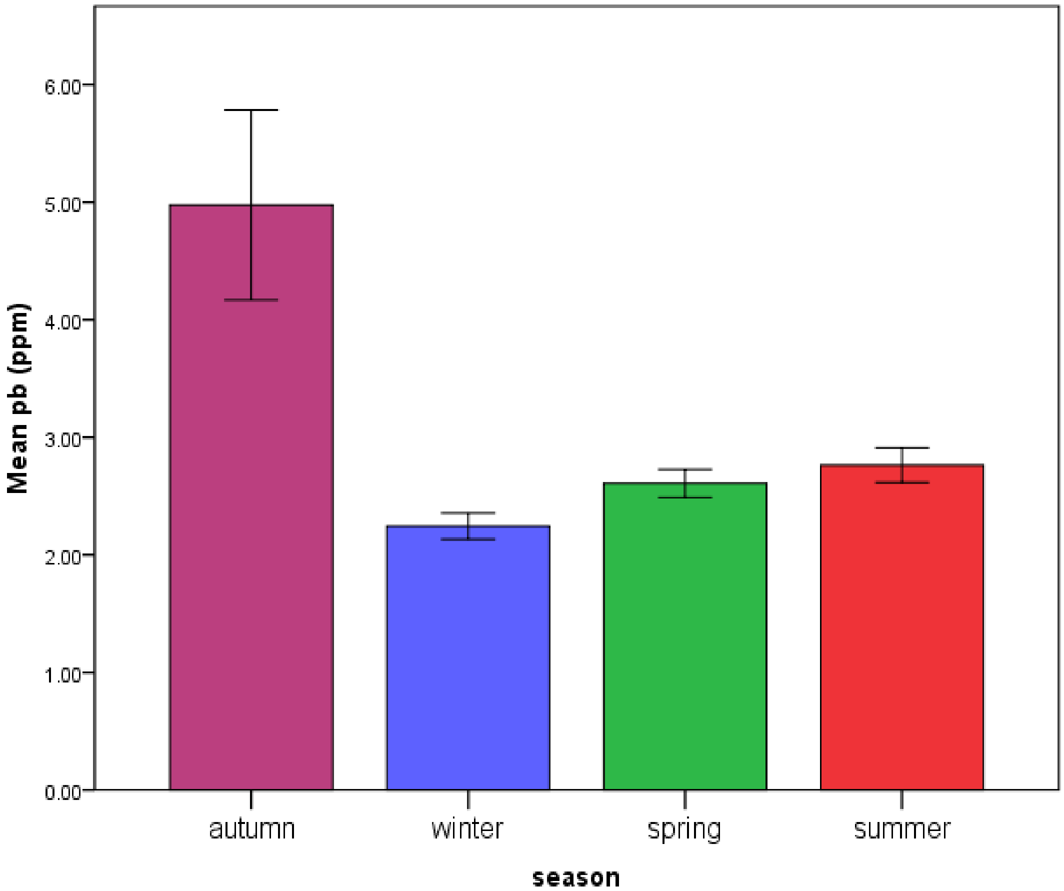


Table 12. ComparativeNi (ppm) in the seasons

seasons	samples	Mean	Std. Deviation	Std. Error Mean
autumn	6	10.5843	5.81121	2.37242
winter	6	4.7792	.48360	.19743
spring	6	2.8435	1.04235	.42554
summer	6	4.8823	2.83150	1.15596

The results showed that there were significant differences between the seasons of the year where the value of the observed level of significance was (0.005) which is less than 0.05.

Fig. 14. Averages with the standard error of Ni (ppm) in the seasons

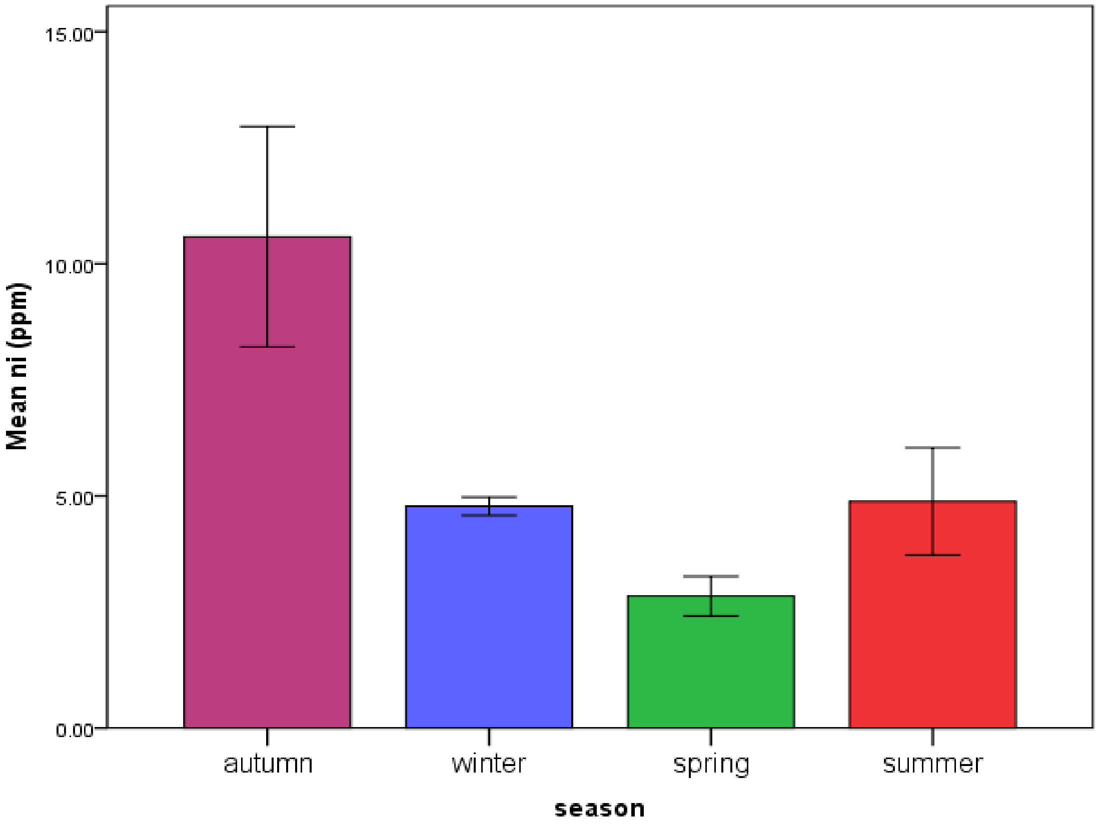


Table 13. Comparison between Mn(ppm) in the seasons

seasons	Samples	Mean	Std. Deviation	Std. Error Mean
autumn	6	95.8513	18.79539	7.67319
winter	6	10.6165	.47304	.19312
spring	6	13.3643	2.24244	.91547
summer	6	11.9416	.44288	.18080

The results showed that there were significant differences between seasons of the year where the value of the observed level of significance was P-value equal to (0.000) which is less than 0.05.

Fig. 15. Averages with the standard error of Mn(ppm) in the seasons

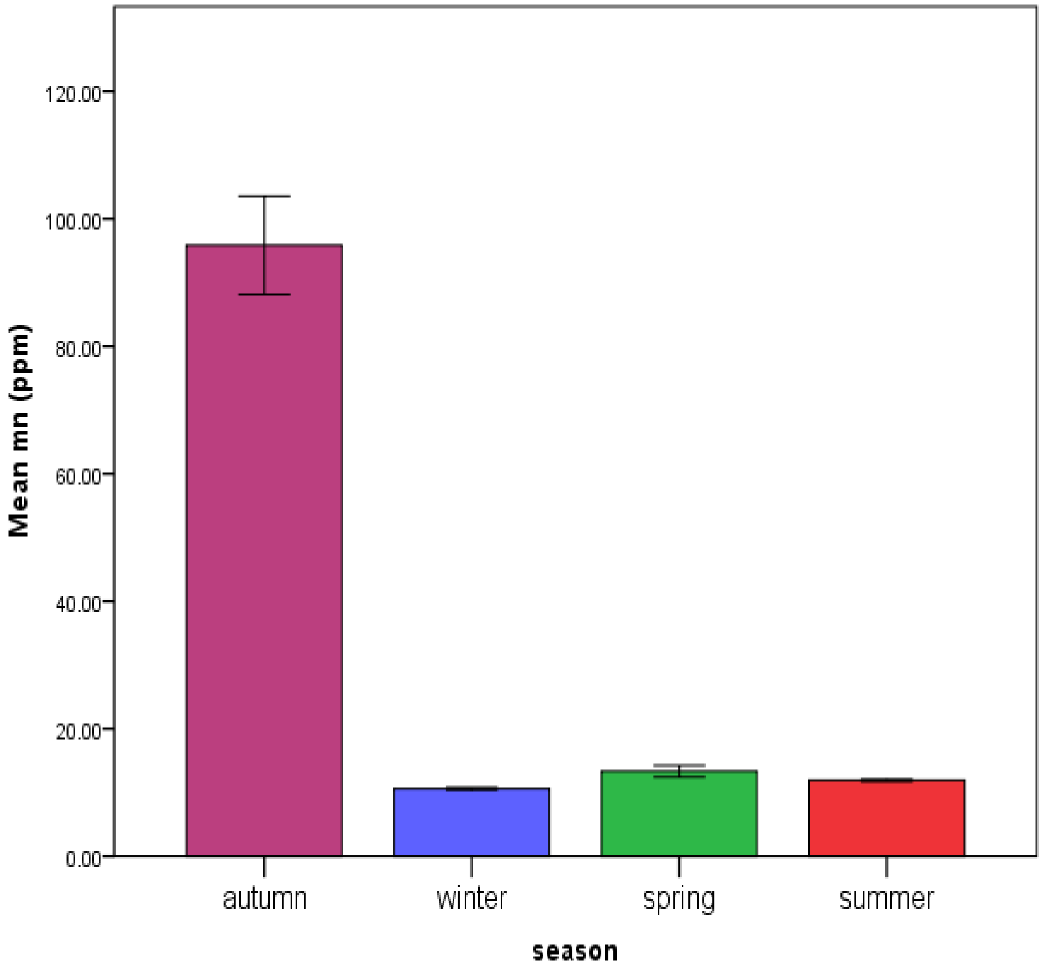


Table 14. Comparison between Fe (ppm) in the seasons

seasons	Samples	Mean	Std. Deviation	Std. Error Mean
autumn	6	768.1563	314.21680	128.27847
winter	6	98.5512	3.73646	1.52540
spring	6	101.7975	38.85123	15.86095
summer	6	115.6184	40.62020	16.58313

The results showed that there were significant differences between seasons of the year where the value of the observed level of significance was P-value equal to (0.004) which is less than 0.05

Fig. 16. Averages with the standard error of Fe (ppm) in the seasons

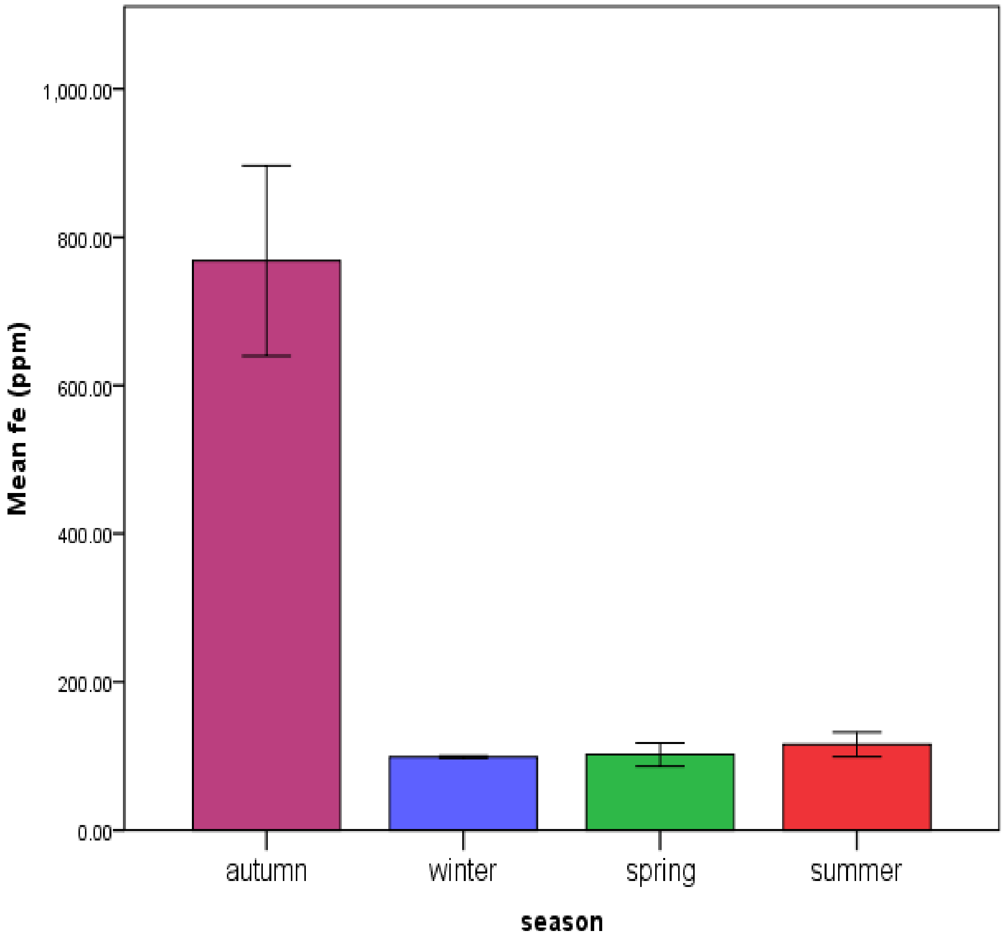
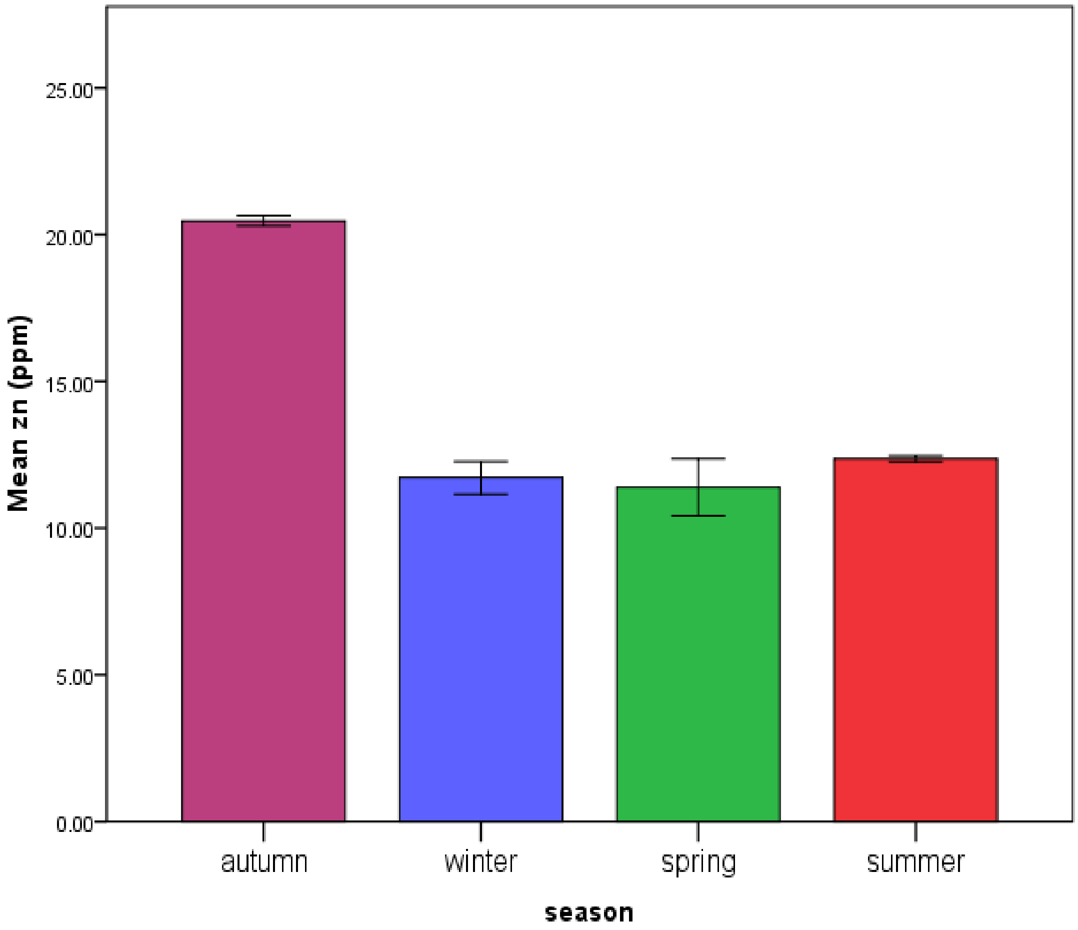


Table 15. Comparison between Zn (ppm) in the seasons

seasons	Samples	Mean	Std. Deviation	Std. Error Mean
autumn	6	20.4714	.42543	.17368
winter	6	11.7163	1.36925	.55900
spring	6	11.3965	2.37380	.96910
summer	6	12.3657	.25299	.10328

The results showed that there were significant differences between the seasons where the value of the observed level of significance was P-value equal to (50.00) which is less than 0.05.

Fig. 17. Averages with the standard error of Zn (ppm) in the seasons



4. Results and Discussion

Tables (1, 2, 4 and 6) describe measures of the two study areas by making a comparison between heavy metals (Cu, Cd, Ni and Fe) in both sites. In order to study the differences between the groups, a Mann-Whitney test analysis was applied. The results showed that there were significant differences between the two regions where the value of the observed level of significance was ($p<0.05$). The tables (3, 5 and 6) describe measurements of the two study areas by comparing between heavy metals (Pb,Mn and Zn). In order to find out the variation between the groups, a Mann-Whitney test analysis was used. The results showed that there were no significant differences between the two regions where the value of the observed level of significance was ($p>0.05$). The results further revealed that all the concentrations of heavy metals in Site 1 were higher than that of Site 2; apart from the concentration of Mn which was (29.3638ppm) at Site 1 and (36.5230 ppm) at Site 2. The increase of the concentrations of heavy metals could be attributed to the fact that Site 1 is near to the city center and port, in which there is a high possibility of city waste discharge and the city's port pollutants in the Black Sea. This will, in turn, increase the concentration of heavy metals in Marine brown alga in Site 1. Moreover, these findings are consistent with those in the study carried out by (Bat et al, 2009). Sinop is the smallest cityin Turkey which is located on the Southern coast of the Black Sea. The total load of its pollution is higher than other cities on the Turkish coast of the Black Sea.

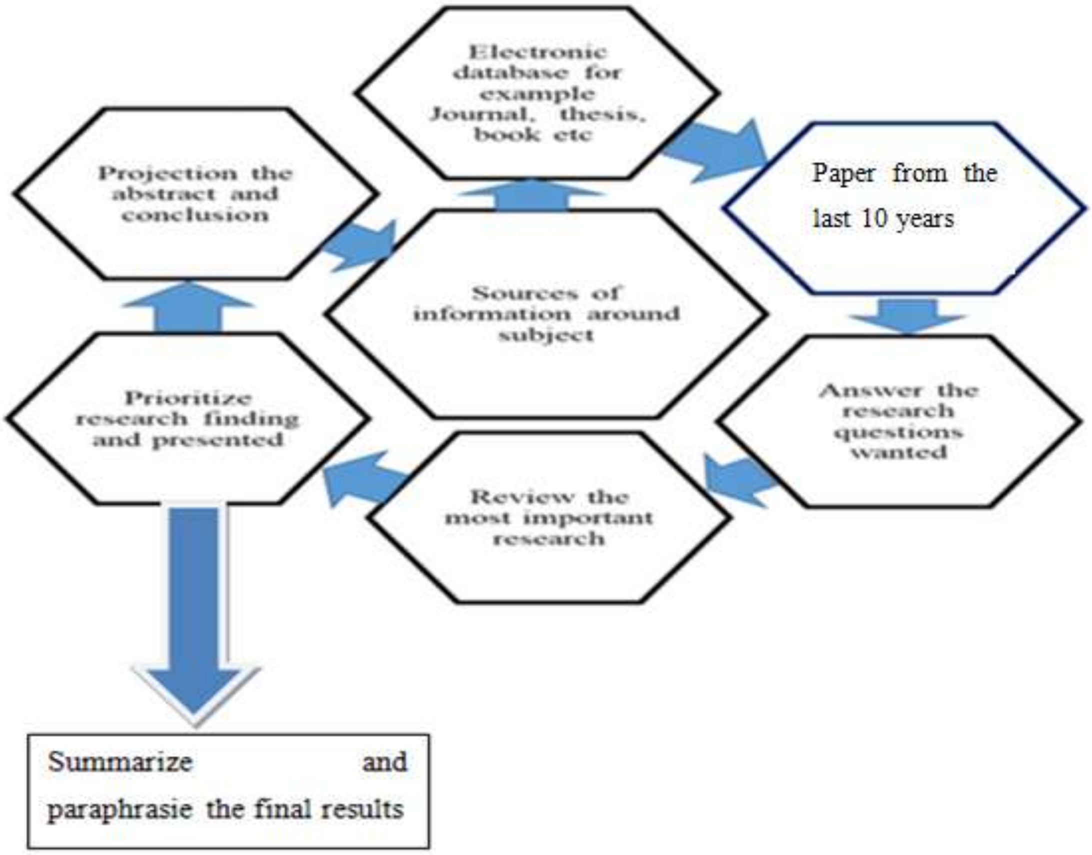


Fig. 18. The research methodology for discuss he research result that used in this section adapted from (Shlibak and Dalla, 2020).

The calculations were conducted according to the flow of a discharge of 52 litres per second. Some of the parameters exceeded the 'Turkish Environmental Regulations' criteria. In the last decade, the local population in Sinop was about 30000. However, the population increased up to 80000 in the summer; since tourists reside near Site 1. Thus, untreated domestic waste and human activity along the coastal zone increase in summer and probably give rise to high levels of pollution. Tables (8, 9, 10, 11, 12, 13 and 14) show descriptive metrics comparing between heavy metals (Mn, Cd, Zn, Cu, Fe, Ni and Pb) during the seasons of the year adopting a Kruskal Wallis test analysis. The results of heavy metals comparison show that there were significant differences in all seasons where the value of the observed level of significance was ($p<0.05$). The results have indicated an increase in the concentrations of heavy metal in autumn than any other season. Such revelations may be ascribed to the fact that, during the year in Turkey, the first rains take place in autumn. Besides, the air contains a great amount of pollutants and these pollutants drop down within the first rain period. Accordingly, the pollutants are carried via rivers and flow in the Black Sea in autumn. The findings further showed relatively higher concentration of Fe than heavy metals in all seasons and within all the study areas. The highest Fe concentrations were (1057.202 ppm) in Site 1 during autumn. Previous studies indicated that quite a large number of algae take on iron by similar fundamental processes as those of higher plants (Sutak, Botebol, Blaiseau, Léger, Bouget, Camadro & Lesuisse 2012). Meanwhile, certain types of species of diatoms as well as unicellular green algae draw on distinct procedures of iron uptake. These unique mechanisms of iron uptake adopted by marine phytoplankton are becoming of wide importance since phytoplankton hold a significant position in the carbon cycle and in primary oxygen production (Morrissey and Bowler, 2012). In addition, the results showed that the Cd concentrations exhibited more decrease throughout the four seasons, than that of other metals in site 2 in spring season (.3918ppm). Finally, several causative factors such as; pH, ligand concentration and type, and multiple sediment components act upon the bioaccumulation of Cd concentrations in brown algae (Tamayo, Guas, Leyte-Vidal & Maccini 2014).

5. Conclusions

The Black Sea is an important ecosystem that constitutes a large part of the total production of fish and crustaceans. In general, increasing industrialization and industrial activities in recent years as well as other intensive activities on the coast and mining activities due to intensive mineral deposits in the Black Sea are constantly threatening the coastline. Hence, this research has presented data on the levels of heavy metals in Marine brown algae in the Sinop coasts (Turkey). On the whole, analyses have shown an increase in the heavy metal concentrations in autumn. The study further showed that the heavy metal concentrations at site 1 are higher than that of site 2. In spite of the findings, it does not pose any form of danger. Therefore, this type of pollution detection studies should be done frequently, and routine reporting should also be conducted in order to take necessary measures to decision mechanisms.

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