Marine Molluscs as Bioindicator for Heavy Metal Levels in the Coasts of Aden –Yemen

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ABSTRACT *Heavy metal levels were investigated in different tissues of bivalves Saccostrea cucullata and two species of gastropods Thais savignyi, Planaxis sulcatus collected from the coasts of Aden in winter and summer. Analysis of metals, Cd,Co, Cr, Cu, Mn and Ni were carried out in soft tissues, using (AAS). The average concentration of heavy metals in T. savignyi :(Cd 8.6,Co4.3,Cr 5.04,Cu 291.8, Mn6.83, Ni 12.58 µg/g). P. sulcatus :(Cd2.39,Co 8.2,Cr10.39,Cu 94.69 ,Mn19.43,Ni 25.95µg/g) S. cucullata: (Cd 5.4, Co 5.14, Cr 7.6, Cu 272.84, Mn 10.8, Ni 12.33 µg/g). The study showed that the highest accumulation of copper and cadmium were found in T. savigny folloewd by S.cuccullata and by P. sulcatus. (T. savignyi >S.cuccullata> P. sulcatus) And accumulation of cobalt, chromium, manganese, nickel) was the highest in P. sulcatus followed by S.cuccullata and by T. savignyi (P. sulcatus>S.cuccullata>T. savignyi).*

Keywords: *Heavy metals; coasts of Aden-Yemen; T. savignyi; S.cuccullata; P. sulcatus.*

Introduction

In marine aquatic ecosystem, mollusks are most frequently used biomonitors for heavy metals, chemical substances and other contaminants in sea water and associated sediments. They feed on every possible food in different ways according to the different forms of molluscs (Szefer et al., 1999). Because of the accumulation effect of some heavy metals, especially through the food chain, their bioavailability needs to be monitored. The investigation of metal concentrations in living organisms may provide information about bioavailability and, the level of environmental pollution (Mol-Aldwila et al., 2017) The increase of sewage and industrial effluents discharged into the Gulf of Aden, As well as oil has seriously threatened the ecosystem. Limited investigation dealing with the presence of various pollutants has been carried out in this area (Ali and Baharoon 2004; Heba et al., 2003). These impacts are mainly caused by human and developmental activities (Rushdi et al., 1994). These activities introduce pollutants to the marine environments and cause the destruction of some special habitats. The most widely recognized issue is the oil- related pollution, where considerable attention has been focused (Al-Shwafi 1997). However, the pollutions areas include the impact of growing industrial and domestic effluents, oil refineries unplanned coastal development as well as various miscellaneous human activities such as fishing, hunting and tourisms, are threatening the marine and coastal ecosystems of the area (Ahmed 2013) This is probably one of potential effects of rising levels of heavy metals.

Materials and Methods

3600 studied specimens were collected from the intertidal and supertited zones from the sampling stations (Fig. 1) From which 2900 individuals of grazing feeder gastropoda, ***Planaxis sulcatus*** (Planaxidae);404 of carnivorous feeder *Thais savignyi* (Muricidae); 296 of filter feeder Bivalvia , *Saccostreacuccullata* (Ostreidae) .In the laboratory, the complete soft tissue of mollusks was removed from the shell and washed with distilled deionized water to remove marine sediment and others impurities.The soft tissue was dried at 80°C till fixed weight. The analytical procedure used to measure the metals Cd,Co,Cr Cu, Mn and Ni; in the molluscs was based on the study Ali and Baharoon,(2000); Otchere (2003) with modification as follows: Three replicate samples of molluscs tissues were weighed accurately 1g and digested with 10 ml of conc. HNO3 ,the samples were then heated for an hour . When the solution cooled, 2 ml of hydrogen peroxide (30 %) (Merck) was added and heated again at 80° C for another 30 min. Finally, the resulting solution was diluted to 100 ml with distilled deionized water. Heavy metal concentrations were analyzed by Atomic Absorption Spectrophotometric (AAS) model (vario 6)

Table (1) Description of sampling sites in the Aden coasts – yemen

No	Location	Latitude(N)	Longitude(E)	Site description
1	Al-Hiswah Coast	12° 49 2.7"	44° 55' 5.9"	Sandy coast, brought large rocks to the coast during the construction of hydroelectric power station adjacent to the coast
2	Al- Kissa Coast	12° 44' 45"	44° 54' 26"	Sandy and Rocky coast, contaminated with sewage waste
3	Al- Ghadir Coast	12° 44' 16''	44° 53' 16''	Sandy and Rocky coast contaminated with sewage waste
4	Kobagen Coast	12° 43' 59"	44° 52' 29"	Sandy and Rocky coast. Watching oil tankers sailing
5	Fuqum Coast	12° 44' 54"	44° 49' 36"	Sandy and Rocky coast. Fishing activities , and a pier for fishermen's boats
6	Amran Coast	12° 45' 52"	44° 44' 52"	Sandy and Rocky coast. quay for fishermen's boats

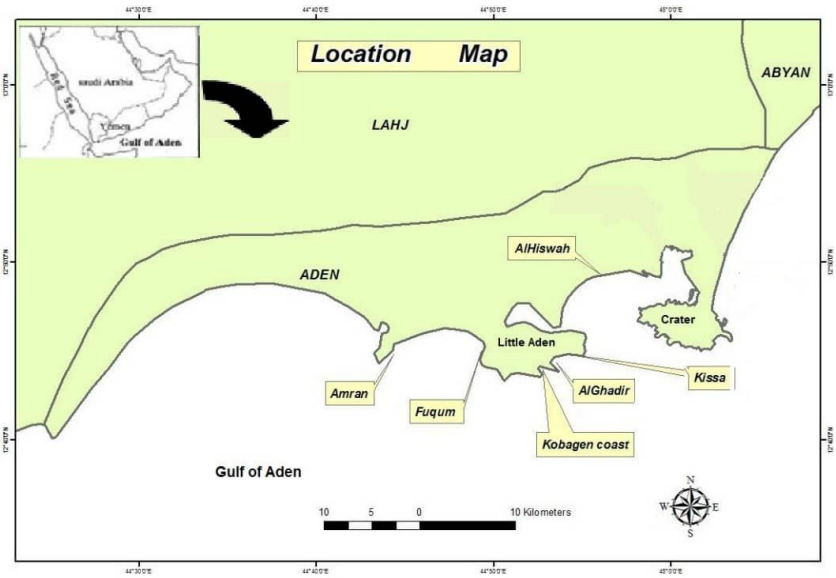

Fig. 1- Study Area and Sampling Sites

Results and discussion

It is clear from the results of the analysis of marine samples (Figure 2, Table 2) that the average concentration ofcadmium in *P. sulcatus* is convergent in all study stations and there are no significant differences between cadmium concentrations in all stations. In addition, in the study of cadmium concentration in winter and summer, the highest concentration (3.15µg/g) showed in the winter's samples of Al-Hiswah Coast and the lowest concentration (1.3 µg/g) was found in Al-Khissa Coast of the summer samples. Also, the average concentration of cadmium *P. sulcatus* in all study stations in winter and summer was (2.39 µg/g). This concentration is less than the concentrations obtained by (Nicolaidou and Nott 1998; Khidkhanet al.,2016) in Greece and Thailand (5.8, 8.14 µg/g) respectively, while Tornimbeni et al. (2013) found the average concentration in their research conducted in Canada (0.70 µg/g). As for *T. savignyi,* there are no statistically significant differences among the stations 5,6, and 2. Also, there is a convergence between 3 and 4, while the average concentration of cadmium in station 1 differs from the rest stations. The highest concentration (13.1 µg/g) was recorded in the samples of winter in station 4 (Kobagen), while the lowest concentration was in Al-Hiswah (5.3 µg/g) in winter, and the average concentration was (8.59 µg/g). Furthermore, the high concentration (35.54µg/g) in a study of Astani et al., (2012) on the coasts of Bandar Abbas- Iran was high compared to the high concentration in the current study. Bedsides, the average total of concentration obtained by Hung et al., (2001) in their study in Taiwan (4.36 µg/g) which was approximately twice as less as the average concentration we obtained in this study.In contrast to the concentration of the *S.cuccullata* samples in the different stations of the study shown in Table 2, it was shown that the average concentration in the stations 1,3,4, is convergent, and also the concentration in the stations 2 and 6. However, the station 5 (Fuqum) was significantly different from the other stations where the average concentration was the highest (See Table 2).

In the analysis of cadmium concentration in *S.cuccullata* tissue in all study stations during the two seasons, it was found that the highest concentration was in winter (14.54 µg/g) from the samples of the Fuqum Coast, while the lowest concentration (3.3 µg/g) was measured in the station 2 (Al-Khissa Coast) in the samples of winter, and the average concentration was (5.4 µg/g). When comparing the average cadmium concentration in the animals of the study, it was observed that *T. savignyi* of the cadmium accumularia was higher than the other two species. This seems to be because of the nature of the *T. savignyi* nutrition, where it feeds on gastropods, bivalves, and barnacles (Watanabe and Young 2006). The average concentration of cadmium in the present study (5.4 µg/g) were convergent is approximates to the average concentration obtained by Iskenderun Bay -Turkey (4.27 µg/g) in Turkmen *et al* ., 2005), but the average concentration of cadmium in the study(de Mora et al.,2004) in both Abu Dhabi and Ras Essa - Oman was higher than the average concentration in the present study. Also, the concentrations obtained by Ali and Baharoon(2002) in Aden (1.32-9.02 µg/g) were lower than those obtained by the present study. The concentration of cadmium was the highest in *T. savignyi* followed by *S.cuccullata* and by *P. sulcatus.*

Cobalt in P. sulcatus tissue was similar in the stations 2 and 5, and the average concentration in the stations 1 and 6 was convergent, while the concentration of cobalt in stations 3 and 4 was different from the concentration of the other stations. The highest concentration of cobalt in *P. sulcatus* tissue in this study was (12.3 µg/g) of the samples collected in the winter from the station 2 (Al –Khissa) and the lowest concentration was (5.53 µg/g) in the summer from the station 6 as shown in Fig. 3. The average concentration of cobalt in winter and summer's samples for all stations was (8.19 µg/g) . These concentrations were convergent with the results of Duysak and Erosy (2014) at the Mediterranean Sea, particularly the results of Autumn Season (2.74- 12.76) but De Wolf et al., (2000) study showed less concentrations (0.41-2.15 µg/g) than those of the present study, and when studying cobalt results in *T. savignyi*, it was observed that the concentrations is little compared to the concentration of *P. sulcatus*. This is the opposite of what was found in the concentration of cadmium in this study and it can be attributed to the physiology of the type in heavy metal metabolism between animals, where the highest concentration was recorded in summer samples (6.39 µg/g) in the station 5 and the lowest concentration of cobalt in *T. savignyi* tissue was found in the summer (2.3 µg/g) Station 4, and the mean of cobalt concentration in all station samples (4.28 µg/g). When comparing the mean of cobalt concentration in the stations of the study, it was found that the concentrations of the stations 1 ,3 and 6,2 were convergent. However, the average concentration of cadmium in the station 5 was less than the concentration of the other stations where it appeared high. Similarly, the cobalt concentration in *S.cuccullata* tissue was observed between the stations 2,3,5 and the stations 4 and 6 were similar in concentration, while the mean concentration of the station 1 was higher and differed from the concentration of all stations. In studying the cobalt concentration in winter and summer's samples, the highest concentration in summer was found in station 1 (Al-Hiswah Coast) (10.52µg/g) and the lowest concentration (3.66 µg/g) from the winter's samples in station 6 (Amran Coast). The mean concentration was (5.14 µg/g) and this is less than the concentration (8.69 µg/g) obtained by Turkmen *et al*., (2005) in Turkey but, greater than the average measured by (de Mora et al., 2004) (0.23, 036 µg/g) in Oman, Abu Dhabi and (Ali and Baharoon, 2002) (0.17- 0.87 µg/g) In Aden.In conclusion the cobalt concentration in *P. sulcatus* is more then in *S.cuccullata* and more less in *T. savignyi.*

Chromium analysis results in tissues *Planaxis sulcatus, Thais savignyi and Saccostrea cuccullata*.Shown in (Figure4 and Table 2) When analysing the average chromium concentrations in *P. sulcatus* tissue in stations 2,3, and 6 no statistically significant differences were observed. In the study of chromium concentrations in the winter and summer samples shown in Figure 4, the highest concentration (14.9 µg/g) has appeared in the winter's samples collected from Al- Hiswah, and the lowest concentration of (6.46 µg/g) was also from the winter samples in station 3 (Al-Ghadir). The average concentration of chromium in *P. sulcatus* tissue studied in winter and summer was (10.39 µg/g).

Table 2: The Average concentration of heavy metals (µg/g dry wt.) in the tissues of Planaxis sulcatus, Thais savignyi and Saccostrea cuccullata.

P. sulcatus							
Location	No.	Cd	Co	Cr	Cu	Mn	Ni
Hiswah	1	2.67	8.25	12.12	117.16	33.85	23.2
Kissa	2	2.02	9.65	8.39	115.42	12.25	28.15
Ghadir	3	2.31	6.47	8.38	89.3	14.16	24.76
Kobagen	4	2.67	7.11	11.17	76.85	11.08	22.51
Fuqum	5	2.25	9.85	13.4	66.725	18.04	30.15

Amran	6	2.45	7.83	8.9	102.71	27.2	26.9
T. savignyi							
Hiswah	1	6.0	2.75	2.76	192.5	24.02	3.9
Kissa	2	7.41	4.63	6.41	313.4	2.11	14.0
Ghadir	3	10.45	2.89	4.25	294.05	4.25	13.95
Kobagen	4	11.35	3.9	5.3	229.98	4.53	12.2
Fuqum	5	8.25	6.77	7.2	273.35	2.9	15.95
Amran	6	8.05	4.77	4.36	447.3	3.2	15.5
S. cuccullata							
Hiswah	1	4.95	7.33	18.05	296.05	16.81	18.95
Kissa	2	3.57	5.3	3.66	204.3	9.47	11.05
Ghadir	3	5.1	5.22	4.66	462.2	20.53	10.05
Kobagen	4	4.93	3.58	7.61	152.2	4.4	9.105
Fuqum	5	10.12	5.65	7.54	205.15	8.95	12.9
Amran	6	3.75	3.8	4.12	317.12	4.67	11.95

This concentration is higher than that found by Tornimbeni*et al*., 2013, but it was lesser than the average chromium concentration (25.2 µg/g) measured by Nicolaidou and Nott (1998) in Greece. As for *T. savignyi*, it was observed the difference of the concentration of chromium in all the stations except the concentration of the stations 3 and 6. The highest concentration of nickel was recorded in *T. savignyi's* tissue (7.73 µg/g) of the samples of the station 2 (Al-Kissa) in the summer season, while the lowest concentration (2.45 µg/g) was observed in the samples of winter at station 1 (Al- Hiswah). The average concentration of chromium in the current study is (5.04 µg/g) and this is small compared with the average concentration (28.5, 17.5 µg/g) obtained by Hung et al., (2001) and Ibrahim and El-Regal (2014) in Taiwan and Egypt, respectively. When comparing the averages of chromium concentrations in *S. cuccullata* samples, it was found that the concentration of the stations 3 and 6 was convergent. This was also observed when comparing the concentration of chromium in the tissues of P. sulcatus and *T. savignyi*.Furthermore, there was convergence in the concentration between the stations 4 and 5. However, the concentration of chromium in the stations 1 and 2 was considerably different, and this difference was observed in the concentration of the two stations in the tissues of *P. sulcatus, S. cuccullata* when analysing the nickel metal. The highest concentration of chromium in *S. cuccullata* tissue was observed in the summer's samples (28 µg/g) and lowest concentration in the winter's samples (8.1 µg/g), both were collected from Al-Hiswah Coast, with an average concentration of (7.61 µg/g). The accumulation of chromium in the tissues of the studied were found high in *P. sulcatus* followed by *S. cuccullata* an by *T. savignyi* .

When comparing the concentrations with the results of Bazzi (2014) in the Sea of Oman during the winter and summer (12.7-27.95 µg/g), there was convergence in the results of the two studies especially in their high concentration, the average chromium concentration in this study (7.61µg/g) was lower than the average chromium concentration obtained by Turkmen, *et al*.,(2005) in Turkey (9.7µg/g)

The results of analysing the *P. sclcatus* shown in (Figure 5 and Table 2) revealed that the average concentration of the copper was convergent in the stations 1,2,6 and the concentrations of the stations 3 and 4 were also convergent. However, the average concertation of the station 5 was different where it presents the least concentration. In studying the concentration of copper in all the stations in both seasons (summer and winter), the highest concentration (190.4 µg/g) was in the samples of summer which were collected from the station 2 (Al-Khissa coast). Besides, the least concentration (40.43 µg/g) appeared in the station 2 and the average concentration of copper was (94.69 µg/g). These concentrations were greater than those obtained in Italy (18.55-32.6 µg/g) by (Conti et al., 2004) and and study (De Wolf et al., 2000) in Belgium (68.22-176 µg/g). The average concentration (94.69 µg/g) is lower than the average measured by Nicolaidou and Nott (1998) in the samples collected from Greece (222.6 µg/g). When comparing the concentration of copper in *T. savignyi* tissue, it was observed that the average concentration varies from one station to another. The highest concentration of copper (519.2 µg/g) was recorded in the summer from samples of Amran Coast (Station 6). In contrast, the lowest concentration (137.75 µg/g) was in the samples of the station 1 which were collected in winter. The average concentration (291.2 µg/g) was lower than that obtained by (Hung et al., 2001) in Taiwan (492). While, the average concentration of copper measured in the samples Timsah Lake in Egypt by Ibrahim and El-Regal (2014) (17.4 µg/g) was very small compared to the concentration of the element in the present study samples.

When comparing the concentration of *S. cuccullata* samples in the different study stations shown in Table 3, it was found that there is no statistically significant differences in the concentration of stations 2 and 5, while the concentrations of the other stations were clearly different. When studying the concentrations of the two seasons (winter and summer), the highest (488.8 µg/g) concentration of copper was recorded in the samples of summer collected from the station 3 (Al-Ghadir Coast), while the lowest concentration (114.4 µg/g) was in winter from the station 4 (Kobagen), and the average concentration was (272.84 µg/g). These concentrations were higher than the studyied concentrations appeared in Oman (Peer et al., 2010) (84.96 - 355 µg/g) and higher than the average concentration measured by Turkmen *et al*.,(2005) in Turkey (64.7 µg/g). The accumulation of copper metal in the tissues of the studied were high in *P. sulcatus* followed by *S.cuccullata* and by *T. savignyi* . According to the studies of Ke and Wang, (2001), the presence of respiratory pigment hemocyanin in the blood of oysters (*S.cuccullata*), where each functional unit of hemocyanin is capable of linking two atoms of copper and four atoms of zinc. These findings help to understand the oyster's high capacity to concentrate copper. However, in this study, *T. savignyi* showed a higher concentration for copper than oyster samples. This can be attributed to the nature of nutrition, where *T. savignyi* feeds on other gastropods, bivalves, and barnacles, including oysters (Watanabe and Young 2006). Therefore, the copper concentration in *T. savignyi* appeared high.

The average concentration of manganese in the *P. sulcatus* tissue shown in Table 2 was similar in the stations 2, 3, and 4. But, the concentration of the Al-Hiswah Staion's sample was significantly higher than that of the other stations. When studying the concentration of manganese in winter and summer's samples, it was found that the highest concentration (36.5 µg/g) was from the samples collected in the summer from Al-Hiswah, while the lowest concentration (8.95 µg/g) was recorded in the summer from the samples of the station 4 (Kobagen) as shown in Figure (6). In addition, the average concentration of manganese in the present study was (19.43 µg/g). In contrast, the concentration of manganese in study (De Wolf et al., 2000) (19.64-113.7) was greater than range the results of the present study. Besides, the average concentration found in Saint-Augustin Lake, Canada (102 µg/g) by (Tornimbeni et al., 2013) and the average concentration measured by (Khidkhan et al., 2016) in Thailand (135.17 µg/g) were higher than the average concentration found in the present study. Nevertheless, the average concentration of the current study(19.43 µg/g) was slightly higher than the concentration measured by Nicolaidou and Nott (1998) in Greece.

The manganese concentrations in the *T. savignyi* tissue shown in Table 2 are highly close in all stations except for the concentration of the sample collected from the station 1 (Al-Hiswah). This is also observed in the *P. sulcatus* sample, possibly due to the high concentration of manganese in the environment of the station 1. When studying the concentration of the manganese in *T. savignyi*, it was observed that these concentrations were small compared to the *P. sulcatus* concentration. Such a concentration arrangement was found in cobalt concentrations.The highest concentration of manganese (28.21 µg/g) appeared in *T. savignyi* tissue was in the samples of Al-Hiswah Station in the summer, and the lowest concentration was recorded in winter (0.91 µg/g) of Al-Khissa samples. The average concentration (6.83 µg/g) was small compared to those found in Iran by Astani et al., (2012) which ranged between (8.67- 57.37 µg/g). As for manganese concentration in *S.cuccullata*, the concentration showed similar in stations 4 and 6. There is also convergent in the concentration of the samples in the stations 2 and 5 and the average concentration of the station 3 was higher and differs considerably from other stations (4,6 and 2,5). The highest concentration was found in*S. cuccullata* (29.25 µg/g) in winter in the samples of Al-Ghadir Station, while the lowest concentration (4.23 µg/g) was found in the summer's samples from Amran Coast. The average concentration of manganese in S. cuccullata tissue was (10.8 µg/g). The accumulation of manganese metal in the tissues of the studied were high in *P. sulcatus* followed by *S.cuccullata* and by *T. savignyi* .

The results of this study were lesser than those found in Oman Sea (29.75-89.21 µg/g) by Bazzi (2014), but the concentrations measured by Ali and Baharoon (2002) at Aden Coats (6.3-19.9 µg/g) wereslightly smaller than those of the present study. In comparison, the average concentration of manganese found in this study (10.80 µg/g) was lower than that obtained by Turkmen et al., (2005) in Turkey (25.37 µg/g). The results of analysing the marine samples (Figure 6, Table 2) the average concentration of nickel in P. sulcatus was convergent in the stations 1,3, and 4. In addition, the average concentration of nickel in the station 2 also approximates with the concentration found in the station 6, but the concentration of nickel in the samples of the station 5 appeared lesser than those of the other stations. The highest concentration (34.2 µg/g) has appeared in winter from the samples of the station 5 (Fuqum), and the least concentration (19.8 µg/g) was in the samples of summer which have been collected from Kobagan Coast (station 4). The average concentration of nickel in the *P. sulcatus* tissue was (25.91 µg/g). When comparing the average concentration of nickel in this study with the results of Tornimbeni et al., 2013) and Amerts and James

(1998), it was observed increasing the concentration of nickel in the present study. However, the average concentration measured by Khidkhan et al., 2016) in Samut Songkhram, Thailand (49.52 µg/g) was twice as high as the average concentration of nickel obtained in this study. On the other hand, as for *T. savignyi* , it was observed the approximation of the concentration in all the stations except the average concentration of nickel in the station 1 (Al-Hiswah). The highest concentration of nickel was recorded in the *T.* savignyi tissue (19.5 µg/g) in the samples of winter which have been collected from the station 6 (Amran), but the least concentration (3.8 µg/g) in the samples of Al-Hiswah (station 1) in winter. The average concentration of nickel in *T. savignyi* tissue (12.58 µg/g) approximates the concentration obtained by Hung et al., (2001) in Tiwan Coasts (12.3 µg/g). When comparing the concentration of nickel in *S.cuccullata* samples, the average concentration of nickel in the stations 3, 4, 5 and 6 appeared convergent, while in the station 1 it was high and low in the station 2. This is the opposite of what was found in the same two stations when analyzing *T. savignyi* tissue. When studying the concentration of nickel in the tissues of *S.cuccullata* in all stations for the seasons; winter and summer, it was found that the highest concentration was (32.93 µg/g) and the lowest concentration in winter was (8.1 µg/g) from the samples of Al-Hiswah Coast (station 1). The mean of nickel concentration in *S. cuccullata* tissue was (12.33 µg/g). The accumulation of nickel in the tissues of the studied were found high in *P. sulcatus* followed by *S.cuccullata* an by *T. savignyi.*

This average concentration of nickel (12.33 µg/g) is higher than those obtained by de Mora et al., (2004) and Turkmen et al., (2005). However, the concentration of nickel (16.33-38.04 µg/g) in the study of Bazzi (2014) in the Sea of Oman are higher than the present study.

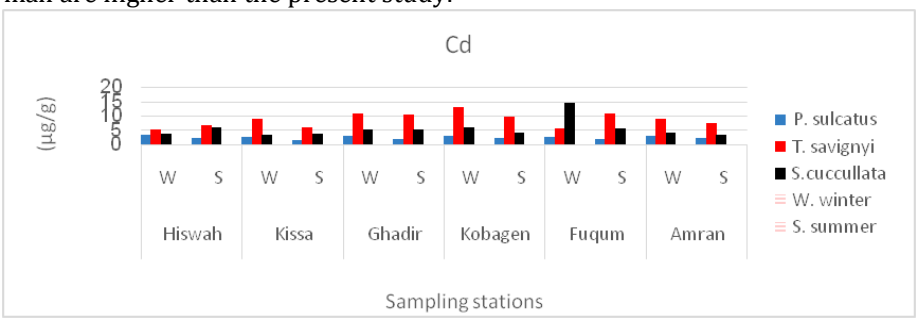

Fig 2. Concentration of cadmium in soft tissue for *P. sulcatusT. savignyi* and *S. cucullata*

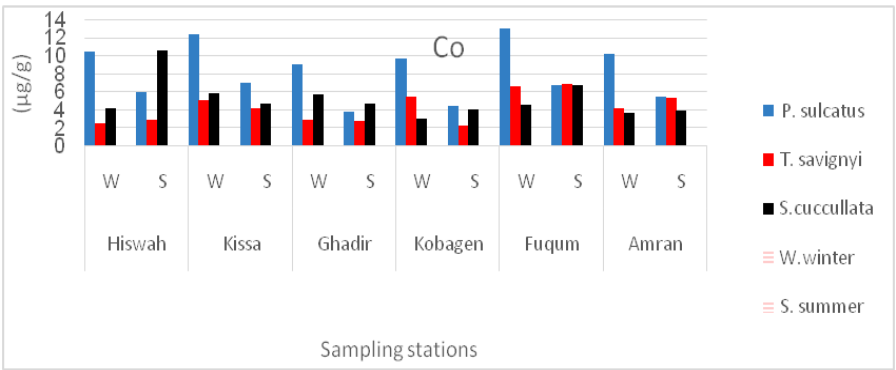

Fig 3. Concentration Cobalt in soft tissue for *P. sulcatus T. savignyi and S. cucullata*

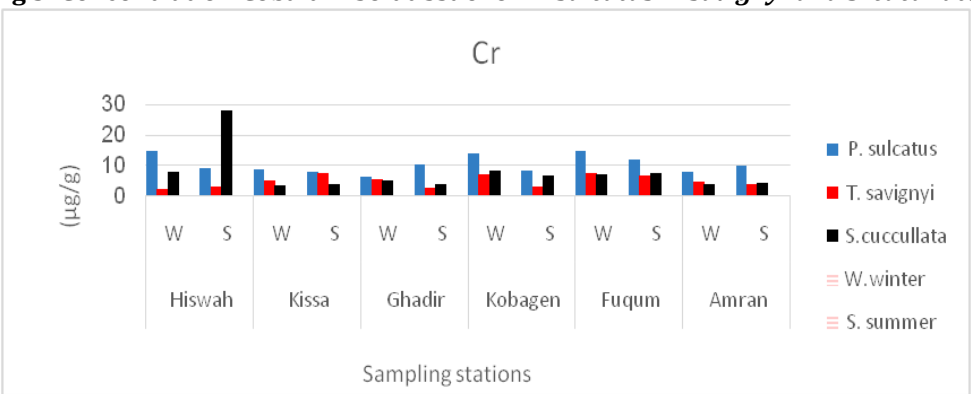

Fig 4. Concentration of chromein soft tissue for*P. sulcatusT. savignyi* and *S. cucullata*

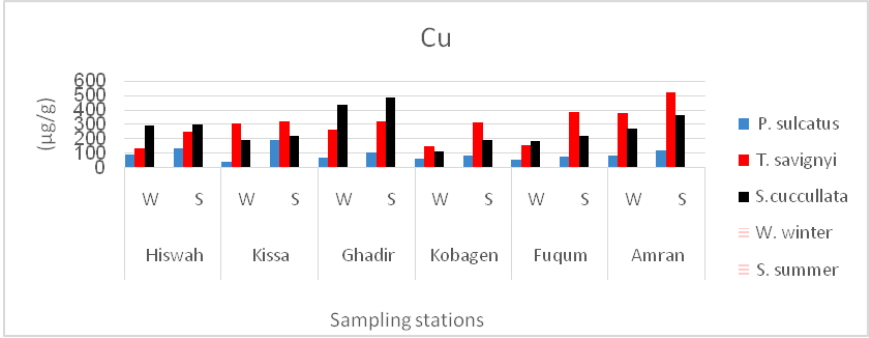

Fig 5. Concentration of Copper in soft tissue for *P. sulcatus T. savignyi and S. cucullata*

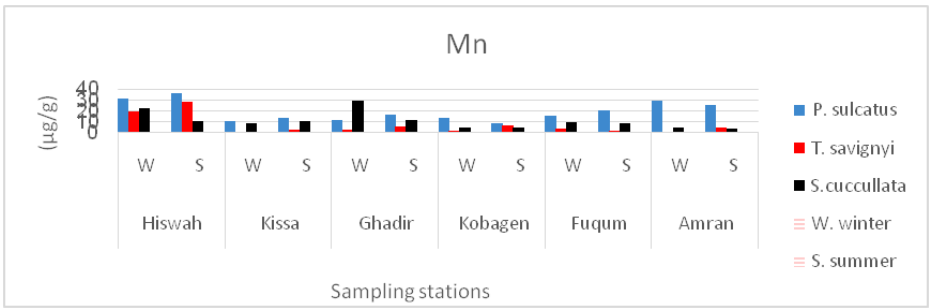

Fig 6. Concentration of Manganese in soft tissue for *P. sulcatus T. savignyi and S. cucullata*

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