Heavy Metal Concentrations in *Padina gymnospora* and *Padina tetrastromatica* Dictyotaceae, Ochrophyta) and Sediments of Bushehr Coastline (Bushehr Province, Iran)

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Abstract

In the present study, in order to investigate and compare the absorption of heavy metals (nickel, cadmium, lead, and copper) in two algae species, *Padina gymnospora* Kuetzing Vickers and *Padina tetrastromatica* Hauck, and in the adjacent sediments along the Bushehr coasts, four sites namely Helilla, Lian, Raphael (burned ship) and Naftkesh were selected for sampling. The sediment and algae sampling was carried out during summer and winter of 2016. After digestion and preparation of samples, an atomic absorption instrument was used to measure the concentration of heavy metals. The mean concentration of Nickel, Cadmium, Lead, and Copper in two samples was measured about 8.91, 1.2, 3.47 and 0.31 $\mu g.g^{-1}$ in *P. gymnospora*, 8.72, 1.15, 3.33 and 0.28 $\mu g.g^{-1}$ in P. tetrastromatica, and 7.93, 1.61, 2.83 and 0.18 $\mu g.g^{-1}$ in studied sediments, respectively. The absorption rate of investigated species and sediments were nickel >copper >lead >cadmium.

Keywords: Bushehr Coastline, Cadmium, Copper, Lead, Nickel, *Padina gymnospora, Padina tetrastromatica*, Sediments.

Introduction

A large part of the environmental pollution crisis is related to marine pollution. Marine pollution includes the introduction of substances or energy by man, directly or indirectly, into the marine environment (including coasts and estuaries), which affected marine living resources and human health. It also prevents fisheries and reduces quality of sea water. This definition is, of course, more related to human induced pollution and has less emphasis on natural pollution (Valikhani et al., 2014). The problem of pollution caused by heavy metals, is due to the stability of them in aquatic environments (Tuzen et al., 2009). Pollution causes biodiversity and living organism abundancy changes, (changes by human or environmental interactions). The frond of seaweed species are highly competent to absorb metals, therefore, they have been regularly used as biological indicators dissolving trace metals in sea water. A tropical seaweed, the brown algae *Padina* spp. have been proven to be capable of tolerating and uptaking trace metals for a long

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time. Among algal groups, brown algae, perform heavy metal absorption more than other macroalgae (Salgado et al., 2005) and among brown algae, *Padina* can be used as an indicator of environmental pollution. Amini et al. (2013) studied the amounts of cadmium, lead, nickel, copper and zinc available in sediments and tissues of *Padina* species and showed a positive and significant correlation between the concentration of most metals in sediments and *Padina* species. Furthermore, in another study, Dadolahi et al. (2011a) investigated the seasonal changes of heavy metals (lead, cadmium, copper, nickel, zinc, and iron) in sediments and eleven marine algae species dominant in Hormozgan. They showed *Padina* species had the most spatio-temporal distribution of absorbed heavy metals and the highest positive and significant correlation with sediments. Therefore, *Padina* were recommended as biological monitoring in this region. Bushehr is located in the southern part of the country, with more than 600 kilometers of coastal border and proximity sensitive coastal and marine habitats. Due to the establishment of industrial factories in coastal and marine areas of Bushehr province, it has always faced serious environmental problems, including the entry of large amounts of hazardous heavy metals into marine environments.

There are many macroscopic algae that can be used analyzing the seasonal and long-term changes of heavy metals. Therefore, this study was conducted to evaluate the absorption of nickel, cadmium, lead, and copper in *P. gymnospora* and *p. tetrastromatica* and the sediments of Bushehr coastal area.

Materials and Methods

Location of sites in the study area

In this study, four sites namely Helilla, Lian, Raphael (burned ship) and Naftkesh were selected for sampling along the tidal zone of Bushehr coast. The selection of sites was based on the amount of human activities in the area, input wastewater, and the presence of algae species (Table 1 and Fig. 1).

Sampling

Sampling was carried out during summer (mid-August) and winter (mid-February) 2016 at full tide time. To determine the maximum tidal time in the selected sites, the Iran Hydrography website (www.Iranhydrography.org) was used to show the tidal condition of coastline in Iran. Due to selected seasons,

Table 1. Location of sampling sites.

Sampling site	Latitude	Longitude	Characteristics
Helilla	28°51'47.5"	50°51'02.0"	remote distance from urban residential area
Lian	28°53'11.3"	50°50'03.4"	shipbuilding activities
Raphael	28°54'55.6"	50°48'45.5"	populated residential area
Naftkesh	28°59'06.0"	50°49'35.6"	Pollution of industrial facilities

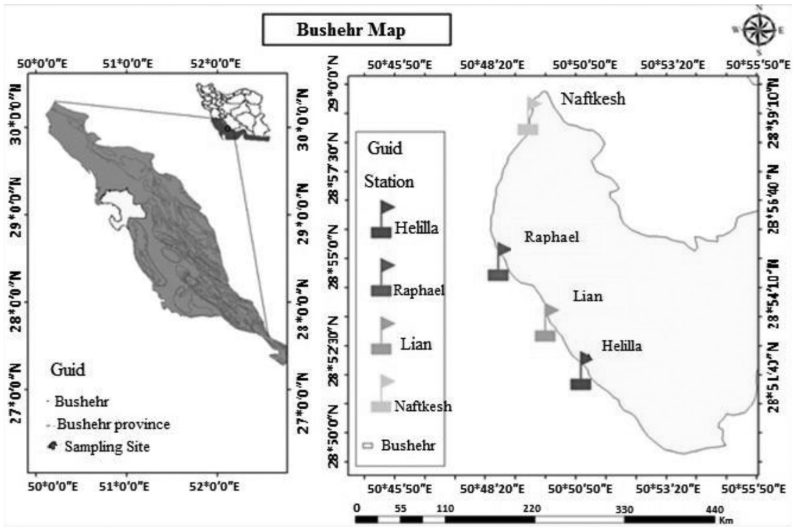

Fig. 1. Location of sampling in the coastline of Bushehr Province.

hot and cold, in the study area, two moderate moderate degree months were selected. Samples were sticking on sea bed, randomly collected by hand from the tidal zone of each site in three replicates (Fig. 1).

To identify the species, morphological characteristics such as color, size and blade shape were recorded then identification of collected species was performed using valid identification keys (Ni-Ni-Win et al., 2013; Hanyuda, 2010; Amini et al., 2013). After rinsing seawater, the collected algae were stored in polyethylene containers preventing any contamination and transferred to the laboratory of Khorramshahr Marine Science and Technology University. Sediment sampling was carried out in three replicates from the surface layer of (0-5 cm) using Van Veen grab with a cross sectional area of 1000 cm^2. The samples were stored in polyethylene containers, kept in a refrigerator at a temperature of less than 4 °C, and transferred to the laboratory.

Preparation of samples for measuring heavy metals

To measure the concentration of heavy metals (copper, lead, nickel and cadmium), of the sediment (the type of sediment was sand) and algae samples rinsed twice with distilled water) were dried in an oven at 105 °C for 24 hours. The samples were ground in a mortar and sieved with a 63 micron sieve and put in polyethylene containers. The chemical digestion of 1 g of algae powdered with 10 ml of concentrated nitric acid was carried out. After one night at room temperature, the samples were placed on a hot plate apparatus at a temperature of 140 °C for 5 hours for complete digestion. Sediment samples digestion was carried out by dissolving 1 g of the sample in 8 ml concentrated Nitrate and 2 ml of HCl (4:1) left at room temperature for 12 hours. Then placed on a hot plate at 140 °C, sediment samples and controls were prepared in three replicates (Alagarsamy, 2006: Tessier et al., 1979).

For both groups of algae and sediment samples, before drying, the containers were removed from the hot plate and 10 ml of nitric acid (10%) was added to the samples. Samples were filtered in volumetric flasks using Wattman filter paper No. 42 and reached to volume of 25 ml with double distilled water and calibration curves of the elements were obtained. With each series of samples, a blank procedure was conducted using all reagents and chemicals to detect any probable contamination. Cadmium, lead, copper, nikel standard solutions (1000 $\mu g.ml^{-1}$) (Spectro ECON) from Chem-lab was used. Digested samples were measured by atomic absorption spectrophotometer (SavantaΣ Savant A & A Sigma; GBC Scientific Equipment Ltd., Dandenong, Australia, using air acetylene flame).

Statistical Analysis

The data obtained in this study were analyzed by SPSS software (version 21) and the diagrams were drawn in Excel. The Shapiro Wilk test was used to test the normal distribution of data. Regarding the normality of the data, one-way ANOVA, Duncan's test, and t-test were used to determine the existence or absence of significant differences between the mean concentration of metals in different sites and seasons. Regression and Pearson correlation coefficient were used to investigate the correlation between the concentration of heavy metals in sediments and the studied algae. Qualitative assessment of heavy metal contents in sediments, was done comparing the concentration of these metals with the values specified in National Oceanic and Atmospheric Administration (NOAA), Canadian Interim Marine Sediment Quality (ISQGs), and United State Environmental Protection Agency (USEPA1). These standards are the most commonly used standards in the world to protect aquatic ecosystems and determine the sediment health in terms of nutrients, organic compounds and concentration of available metals. NOAA has identified two risk levels, Effect Range Low (ERL) and Effect Range Medium (ERM) for metal pollution in sediments. ERL is the level to which 10% of biological communities are at risk, and the ERM is the level that less than 50% of the biological communities are at risk. Probable Effects Level (PEL) is also determined by the Canadian Council of Ministers of the Environment (CCME). The PEL shows the lower limit of the range of chemical concentrations that is usually or always associated with adverse biological effects. Both the (the ERM and the PEL) guideline values are consider to represent the concentration which toxic effects are usually or frequently observed. (De Mora and Sheikholeslami, 2002).

Results

Table 2 and 3 show the statistical results obtained from the measurement of heavy metals in sediments and *Padina* species in the study area. Table 2 shows the average concentration of heavy metals in sediments, *P. gymnospora* and *P. tetrastromatica*, in three sites during February and August (Fig. 2). The results of lead accumulation in algae show a significant but positive correlation between the content of lead and the average accumulation of copper in two *padina* species (N=24, r=0.50, P ≤0.05). The results of lead accumulation in algae show a significant but negative correlation between the amount of this metal and the average accumulation of copper in two *padina* species (N=24, r= -0.47, P≤0.05). The correlation between the cadmium concentration of sediments and the average content of nickel in two algae species is positive (N=24, r=1.0, P ≤0.01). The comparison of concentration of metals showed no significant difference between summer and winter samples (P≤0.05).

Also, despite the similarity of pollutant resources and the lack of similarity in spatial, industrial, and population characteristics of the sites, no significant difference was ob-

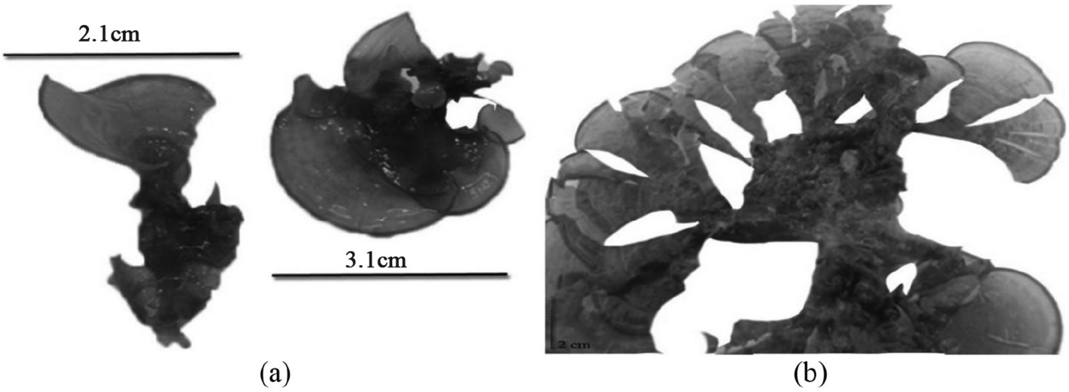

Fig. 2. Habitat of marine brown algae: a) P. *gymnospora* and b) *P. tetrastromatica*.

Table 2. The mean concentration of heavy metals (mean ± standard deviation) in sediments (μg.g^{-1}) in winter and summer.

Season	Cadmium	Lead	Copper	Nickle
Winter	0.2 ± 0.1	1.58 ± 1.17	2.9 ± 0.23	7.01 ± 1.17
Summer	0.17 ± 0.09	1.63 ± 0.17	2.76 ± 0.55	8.85 ± 2.65

Table 3. The comparison of heavy metal concentration (mean ± standard deviation) in two studied algae species (μg.g^{-1}) in winter and summer.

Species	Season	Cadmium	Lead	Copper	Nickle
P. gymnospora	Winter	0.36±0.12	0.99±0.46	3.47±0.41	9.88±2.38
	Summer	0.27±0.09	1.2±0.94	3.88±2.01	7.95±1.3
P. tetrostromatica	Winter	0.32±0.11	0.93±0.47	3.33±0.39	9.68±2.26
	Summer	0.25±0.09	1.15±0.95	3.7±2.09	7.76±1.2

served between the concentrations of heavy metals measured in different collection sites ($P \leq 0.05$).

Differences in the lead content of algae samples were found to be roughly the same in two seasons; however, despite no significant difference in sediment samples of Lian site in winter (3.25 ± 1.8 µg.g^{-1}), it was significantly higher than other sites in summer (Fig. 3). Comparising of heavy metal (nickel, copper, lead and cadmium) concentrations in sediment of Bushehr coastlines with the other results obtained are presented in Tables 4 and 5.

In order to evaluate the heavy metals of sediments, the concentration of heavy metals was compared with the values in some international standards (Table 6).

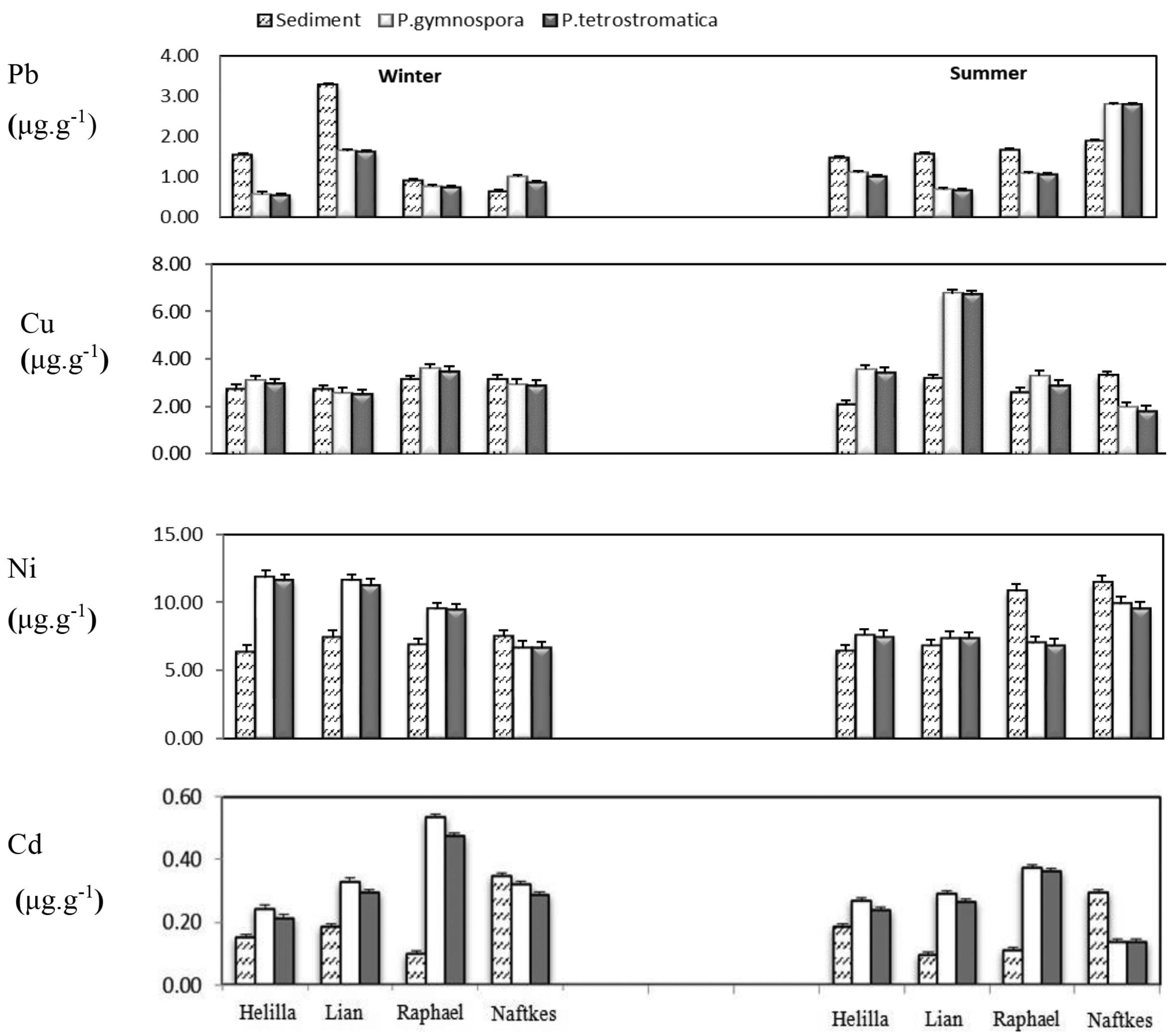

Fig. 3. The average concentration of heavy metals in sediments and *P. gymnospora* and *P. tetrastromatica* in the tidal zone of Bushehr coast in the summer and winter season (the error bars represent the standard deviation).

Table 4. A comparison between the results of the heavy metals concentration ($\mu g.g^{-1}$) in the sediments of Bushehr coast and the sediments of other points of the Persian Gulf.

Reference	Cd	Ni	Pb	Cu	Sampling sites
Amini et al., 2013	5.54	18.9	20.05	27.1	Nayband
Amini et al., 2013	1.37	15.1	5.22	16.4	Bostaneh
Dadolahi et al., 2011 b	4.15	18.1	23.1	9.4	Strait of Hormuz
Alahverdi et al., 2012	10.5-4.6	23.2-17.8	69.5-18.3	5.14	Bushehr
This study	0.18	7.93	1.61	2.83	Bushehr

Table 5. A comparison between the results of the heavy metals concentration ($\mu g.g^{-1}$/dry) in *padina* species of Bushehr coast and other points of Persian Gulf.

Reference	Taxa	Cd	Ni	Pb	Cu	Sampling site
Tuzen et al., 2009	*P. pavonica*	2.27	5.2	28.6	3.35	Black Sea
Allahverdi et al., 2012	*P. tetrastromatica*	0.6	3.54	5.15	3.88	Boushehr Province
Dadolahi et al., 2011 a	*P. pavonica*	5.1	44.4	18.9	10.9	Hormozgan Province
Buo-Olayan et al., 1996	*P. gymnospora*	-	7.25	20.8	4.65	Kuwait Coast
	P. tetrastromatica	-	9.1	2.2	1.75	
This study	*P. gymnospora*	0.31	8.91	1.2	3.47	Boushehr Province
	P. tetrastromatica	0.28	8.72	1.15	3.33	

Table 6. Comparison between heavy metal concentrations in the sediments of Bushehr coast and the global standard values ($\mu g.g^{-1}$).

Heavy Metal	USEPA1 (Bowen, 1979)		Canadian Environmental Standard (CCME)		American Sediment Quality NOAA (Long et al., 1995)		This study
	HAL	LAL	ISQGs	PEL	ERL	ERM	
Cu	270	2	18.7	108	34	270	2.83
Pb	218	2	30.2	112	46.7	218	1.61
Cd	9.6	0.04	0.7	4.2	1.2	9.6	0.18
Ni	50	3	15.9	42.8	20.9	51.6	7.93

Discussion

The results of this study showed a decreasing trend of heavy metal concentration (in order to nickle> copper> lead> cadmium) in sediments and *Padina* species in Bushehr coastal zone. This order shows that the concentration of nickel in sediments and algal species in the studied area is higher than other heavy metals (Tables 2, 3 and Fig. 2). Several studies in the Persian Gulf, the Gulf of Aden, Hormoz, and Nayband show a similar pattern with the mentioned order of heavy metals concentration (Al-Shwafi et al., 2008; Dadolahi et al., 2011b, Amini, 2013). According to many studies, the reason for the high concentration of this element can be due to human activities such as urban and industrial wastewater, the traffic of ships, boats and oil tankers, and petroleum (De Astudillo et al., 2005; El Tokhi et al., 2008). Differences in the lead content of algae samples were found to be roughly the same in two seasons; however, despite no significant difference in sediment samples of Lian site in winter (3.25 ± 1.8 $\mu g.g^{-1}$), it was significantly higher than other sites in summer as showed in Fig. 2.

The sources of lead input to the coasts include the traffic of boats, coloring operations and lead release from the vessels' paint (Luoma and Rainbow, 2008) and wastewater from the metallurgical and chemical industries (Mostafa, 2004). There was no changes between the two seasons sediments sampling for Cu accumulation. However, in summer, the content of copper in *P. gymnospora* and *P. tetrastromatica* was calculated 6.72 ± 2.32 $\mu g.g^{-1}$ and 6.72 ± 2.18 $\mu g.g^{-1}$, respectively (Fig. 2). In study conducted by Amado et al. (1996) the content of copper in *Padina* was reported between 1 to 1.5 $\mu g.g^{-1}$ in unpolluted areas; however, in contaminated areas it is reported to be 32 $\mu g.g^{-1}$ in *P. gymnospora* and 8 to 18.3 $\mu g.g^{-1}$ in *P. tetrostromatica*. The average concentration of cadmium, except in Naftkesh site, in both seasons, was higher in the algae samples in compare to sediment samples (Fig. 2). Cadmium is a contaminant element which enter to the sea as a result of using chemical fertilizers in agriculture, contaminated atmospheric precipitations, effluent from industrial plants and industrial activities or mines; thus, the content of cadmium was measured to be low in the sediment samples of the sites studied, which could be related to the high solubility of Cd in water (Amini et al., 2013).

Also in Qari et al. (2015) investigated content of heavy metals in two algae *P. pavonica* and *P. tertastomatica* in two different coasts of Karachi seasonally, and the results indicated that the content of cadmium in two *Padina* species was lower than nickel, copper and lead, which is consistent with the data of this research. Comparising of heavy metal (nickel, copper, lead and cadmium) concentrations in sediment of Bushehr coastlines with the other results obtained are presented in Tables 4 and 5.

The results showed that the mean of metal concentrations studied in Bushehr coast is lower than the values of NOAA and Canadian sediment quality guidelines; however, it is higher than the lowest standard warning level of the American Environmental Protection

Agency. Although, the lead concentration is lower than the allowed level of this standard, there is a striking distance to reach this value. Marine algae, by collecting heavy metals in their tissues, are used as an indicator for metal pollution in coastal and estuarine waters. Among algae groups, brown algae uptake heavy metals more than other macro algae (Salgado et al., 2005).

Due to the presence of alginates in the cell wall, these algae perform better absorption. The metal absorption by plant is through combining the metal with alginates in the cell wall. Based on Donnan equilibrium theory, an ion exchange occurs between the calcium in the gelatin around the cell and the bivalent metal in the solution, binding selectively to these metals (Xie, 2007). The selection of *Padina* as a biological indicator has been due to the fact that this genus has the potential to uptake more metals than other macroscopic genera (Rai et al., 1981; Ferletta et al., 1996). Also Dadolahi et al. (2011a) reported that among the brown algae, *Padina* was found in all seasons and in all sites of the Hormozgan coast and accumulated the highest concentrations of copper, nickel, and cadmium. Chakraborty et al. (2014) found that *Padina gymnospora* and *Dictyota bartayersieana* had more potential to absorb heavy metals. The study of four algae and water to uptake heavy metals on the Black Sea Coast of Sudan showed that there was no significant difference between the absorption of heavy metals in water and algae, and the two genera, *Padina* sp. and *Cystoria* sp., were better indicator for the heavy metal absorption than *Sargassum* sp. and *Turbinaria* sp. (Aya et al., 2017). As shown in Figure 2 both species showed well spatial and temporal changes in the concentration of heavy metals, indicating the potential of *Padina* algae for the use in biological monitoring studies. This is consistent with the results of the study by Momboya et al. (2007).

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