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ORIGINAL PAPER

Assessment of total and organic vanadium levels and their bioaccumulation in edible sea cucumbers: tissues distribution, inter-species-specific, locational differences and seasonal variations

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Abstract The objective of this study is to investigate the levels, inter-species-specific, locational differences and seasonal variations of vanadium in sea cucumbers and to validate further several potential factors controlling the distribution of metals in sea cucumbers. Vanadium levels were evaluated in samples of edible sea cucumbers and were demonstrated exhibit differences in different seasons, species and sampling sites. High vanadium concentrations were measured in the sea cucumbers, and all of the vanadium detected was in an organic form. Mean vanadium concentrations were considerably higher in the blood (sea cucumber) than in the other studied tissues. The highest concentration of vanadium (2.56 μg g^{-1}), as well as a higher degree of organic vanadium (85.5 %), was observed in the *Holothuria scabra* samples compared with all other samples. Vanadium levels in *Apostichopus japonicus* from Bohai Bay and Yellow Sea have marked seasonal variations. Average values of 1.09 μg g^{-1} of total vanadium and 0.79 μg g^{-1} of organic vanadium were obtained in various species of sea cucumbers. Significant positive correlations between vanadium in the seawater and V_{org} in the sea cucumber ($r = 81.67$ %, $p = 0.00$), as well as between vanadium in the sediment and V_{org} in the sea cucumber ($r = 77.98$ %, $p = 0.00$), were observed. Vanadium concentrations depend on the seasons (salinity, temperature), species, sampling sites and seawater environment (seawater, sediment). Given the adverse toxicological effects of inorganic vanadium and positive roles in controlling the development of diabetes in humans, a regular monitoring programme of vanadium content in edible sea cucumbers can be recommended.

Keywords Vanadium · Sea cucumber · Tissues distribution · Inter-species-specific differences · Locational differences · Seasonal variations

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Introduction

Vanadium is an essential element for many living organisms, including humans (Erdem et al. 2011).

Vanadium can exist in many oxidation states from −1 to +5, but it is most commonly found in the +4 and +5 states (Burba and Willmer 1986). In certain compounds, the oxide and a number of other salts of vanadium are toxic to living organisms at mg L^{-1} level. Foods contain low vanadium concentrations, but the estimated daily intake ranges from 10 to 60 mg (Evangelou 2002). An important role of vanadium in marine organisms is of an active centre of enzymes, such as the vanadium bromoperoxidase of some ocean algae (Clague et al. 1993), but its precise role and mechanism in mammals remains to be studied.

Vanadium was reported to mimic the metabolic effects of insulin in rat adipocytes (Heyliger et al. 1985), and vanadium was observed to act in an insulin-like manner in muscle and liver, as well (Gil et al. 1988; Brichard et al. 1992). Compounds of the trace element vanadium were shown to improve glucose homoeostasis and insulin resistance in animal models of type 1 and type 2 diabetes mellitus (Srivastava and Mehdi 2005) as well as in a small number of diabetic human subjects (Kobayashi and Olefsky 1979; Ramachandran et al. 2003, 2004a, b). Most of the vanadium complexes to date investigated for their possible antidiabetic activity were poorly absorbed in their inorganic forms and required high doses, which have been associated with undesirable side effects (Sakurai et al. 2000; Ramachandran et al. 2003; Pillai et al. 2013). Subsequently, various organo-vanadium compounds have been exploited for their anti-diabetic properties without overt signs of toxicity (Clark et al. 2014).

Food is the major source of exposure to vanadium for the general population (Barceloux and Barceloux 1999; large amounts of vanadium ions are observed in small number of organisms. Henze et al. discovered high levels of vanadium in the blood cells of an ascidian approximately 100 years ago (Henze 1911). The vanadium concentration in this species can reach 350 mM, which is 10^7 times as much as the concentration found in seawater (35 nM) (Heyliger et al. 1985). Vanadium-binding proteins (vanabins), which can accumulate high levels of vanadium, were first discovered by Michibata et al. (Ueki et al. 2003). The vanabins were then isolated from ascidians, and their features and mechanism of vanadium accumulation were studied (Ueki et al. 2007, 2008, 2009; Kawakami et al. 2009).

The majority of the organisms taken from aquatic environments for human consumption are derived from marine ecosystems. Heavy metal pollutants in the marine environment have been shown to bioaccumulate in marine organisms, and many marine animals can accumulate heavy metals. These accumulated metals can originate from food, water or sediment, and their relative importance varies with the metal and the nature of the organism (Jennings and Rainbow 1979). Vanadium accumulation in invertebrates has not been evaluated to date. Sea cucumbers are marine invertebrates of the phylum of Echinodermata that have traditionally been used as a healthy food and medicinal resource since ancient times (Chen 2004). Previous studies have confirmed that it contains various types of bioactive materials, such as sulphated polysaccharide, saponin, collagen and glycosylsphingolipid (Takashi et al. 2005; Wang et al. 2012), and all sea cucumber species were rich sources of Na, Mg, Ca, Fe, Cu, Se, Cr and V for human consumption (Wen and Hu 2010). It has been shown in our previous studies (Liu et al. 2012) that sea cucumbers cultured in spring contain vanadium in the body walls at a high concentration of 1.83–4.58 μg g^{-1}. This is almost comparable with that in most marine organisms, such as cetaceans (*Tursiops truncates*, muscle, 0.12–0.36 μg g^{-1}), fish (*Neogobius melanostomus*, muscle, 0.048–0.027 μg g^{-1}), crustaceans (*Penaeus semisulcatus*, soft tissues, 0.071 ± 0.009 μg g^{-1}) and cephalopods (*Octopus vulgaris*, whole tissues, 1.0 ± 0.2 μg g^{-1}) (Bellante et al. 2009; Miramand and Guary 1980; Anan et al. 2005). Sea cucumbers have been reported to be vanadium accumulators (WHO 1984), and they are a potential source of natural organic vanadium. To determine the key role of vanadium, it is worthwhile to look for the potentially active form of vanadium in sea cucumbers and test its antidiabetic activity to circumvent the toxicity. In our work, we aim to have a better understanding of the biological form of vanadium in edible sea cucumbers and the bioaccumulation effect of this metal.

Materials and methods

Sample collection

For analyses of vanadium in different sampling seasons, the sea cucumbers *Apostichopus japonicus* cultured in the sampling sites of Dalian City and Weihai City in China were harvested in May and

November 2012. For analyses of vanadium in different sampling sites and seawater environments, the sea cucumbers *A. japonicus* were cultured and harvested in eight coastal habitats of three seawater environments (Bohai Bay, Yellow Sea and East China Sea) in China in November 2012. All sea cucumbers *A. japonicus* cultured for 2 years and weighing 110–130 g were harvested. This measure was taken to reduce possible variations in metal concentrations due to size and age. Samples were stored in an iced cooler during transportation. After arrival to our laboratory, they were maintained in an aquarium that contained circulating natural seawater at 18 °C until use.

For analyses of vanadium in different species of sea cucumbers, fourteen species of edible sea cucumbers from two locations (southern Gulf of California, Xisha Waters of the South China Sea), natural sun dried, which were harvested in spring and same weight in different individuals of the same family, including five members of Stichopodidae family, six members of Holothuriidae family, one member of Cucumariidae family, one member of Psychopotidate family and one member of Caudinidae family, were purchased from an aquatic product market, Nanshan Market, in Qingdao, China. The specimens of sea cucumbers were identified by Professor Liao Yulin from the Institute of Oceanography Chinese Academy of Sciences (Qingdao, China).

Sediment and seawater samples were taken at the same sites with sea cucumbers for vanadium content analysis. Seawater samples (approximately 50 mL) and sediment samples were collected 10 m under the sea. On arrival at the laboratory, seawater samples were stored in a 4 °C refrigerator and analysed within 1 week.

Samples preparation

Tissue samples of *A. japonicus* sea cucumbers were taken from the mouth, retractor muscles, intestine, gonad, capillaries, body wall and respiratory tree for vanadium analysis. Soon after they were caught, they were dissected (using high quality stainless steel instruments on a clean glass working surface) to separate mouth, respiratory tree, intestine, gonad, blood, body wall and retractor muscles tissues. These samples together with the dissected samples were immediately deep-frozen (at −80 °C) and transported to the laboratory and preserved at −25 °C until analysis. Fourteen species of natural sun-dried, edible sea cucumbers were grounded into powder. For the determination of organic vanadium (V_{org}) levels in the sea cucumbers, sea cucumber powder was dissolved in ultrapure water and transferred into the dialysis bag (molecular weight cut off 3.5 kDa) after freeze–thaw cycle treatment and then dialysed for 48 h at 4 °C, with dialysis fluid replacement by ultrapure water every 12 h. The sea cucumbers after dialysis were dried at 110 °C until they attained constant weight.

Sediment and seawater samples were taken at the same sites with sea cucumbers for vanadium content analysis. Sediment samples were freeze-dried, passed through a 250-mesh nylon sieve and subsequently stored in polypropylene bottles at room temperature. These sediment samples were dried at 110 °C until a constant dry weight was obtained. Seawater samples were filtered through a 0.45-μm microporous membrane; the pH was adjusted to <2 with concentrated nitric acid. A pre-treated seawater sample volume of 800 mL, containing vanadium ions, was transferred into a stoppered flask; 10 mL of acetate buffer solution and a volume of PAN solution (0.25 %) were added. After fast shaking, a mass of active carbon was added and the mixture was shaken again for a certain time. After filtering, the residue of active carbon was transferred to an Erlenmeyer flask and dried at 110 °C until a constant dry weight was obtained (Pekiner et al. 2014; Ferreira et al. 2002).

Analytical procedure

All samples (0.3 g) were digested with 15 mL of a mixture of 4:1 (v/v) nitric acid and perchloric acid under a laminar flow hood and immersed in the ultrasonic bath at room temperature for 15 min. Digestion was performed by using a hot block system at 80 °C for 20 min, 100 °C for 20 min, 160 °C for 30 min and 190 °C for 30 min. After dilution to 10 mL with 0.1 mol L^{-1} EDTA as a matrix modifier and 1 % HCl (v/v), using aqueous (1 % HCl) calibration, the analyte was injected into the GFAAS for analysis. Before analysis, the samples should be filtered through a 0.45-μm membrane filter, and sample blanks were prepared in a similar manner as the field samples. The digestion procedure was done in triplicate for each sample, and all vanadium concentrations were determined on dry weight basis as μg g^{-1}.

Graphite furnace atomic absorption spectrometry (GFAAS) combined with ultrasound-assisted digestion

(Filik and Aksu 2011) was applied for the determination of V_T (sea cucumber, sediment and seawater) and V_{org} (sea cucumber). An AA-6800F atomic absorption spectrometer (Shimadzu, Japan) equipped with a GFA-EX7 graphite furnace atomizer, a background absorption corrector with a deuterium lamp and a self-reverse system, a pyrolytic graphite tube with an integrated platform and an ASK-6100 automatic sampler were used for the determination of the analyte elements. Pyrolytic graphite coated tubes obtained from Shimadzu and V hollow cathode lamp (Beijing Shuguangming Electronic Lighting Instrument Co., Ltd.) were used throughout the experiments. In GFAAS, argon was used as an insert purging gas during the drying, ashing and cleaning stages and its flow was interrupted during atomization. The absorption signals detected during atomization were recorded as peak heights. In this method, 20 µL blank, standard, sample blank and sample solutions were injected into the graphite tube through an autosampler and heated according to the temperature programme based on our previous orthogonal L_9 $(3)^4$ test design and the optimal operating parameters for GFAAS were listed in Table 1.

Sample preparation (method) and analytical (instrument) quality control (QC) included the analysis of duplicate samples and spiked samples. All analyses were undertaken at least in triplicate on each sample and the mean values calculated. Instrumental reproducibility for vanadium analysis averaged 3.2 % relative standard deviation (% RSD), with a range of 1.01–6.82 %. Method reproducibility for ten duplicate samples, split in the field, averaged 2.1 % RSD and ranged from 0.94 to 4.35 %. The limit of detection (LOD = 1.44 ng mL^{-1}), based on a signal-to-noise ratio of 3, was calculated as $3S_b/S$ (S_b: standard deviation of the blank signals; S: slope of calibration curve). Vanadium concentrations in all samples were well above this detection limit. A known amount of a vanadium standard solution was added as an internal standard to assess matrix effects. All analyses were undertaken at least in triplicate on each sample and the mean values calculated. The relative recovery of vanadium after spiking was 94.2–105.4 %, showing no matrix interferences.

Table 1 Operating parameters for GFAAS

Parameters				
Lamp current (mA)				10.0
Wavelength (nm)				318.4
Slit (nm)				0.5
Integration time (s)				5
Sample volume (µL)				20
Cycle	**Temperature (°C)**	**Hold (s)**	**Heating mode**	**Ar flow (L min^{-1})**
Temperature programme				
Drying	150	30	RAMP	1.0
	250	10	RAMP	1.0
Ashing	900	10	STEP	1.0
	900	10	STEP	1.0
	900	3	STEP	0.0
Atomization	2700	3	STEP	0.0
	2700	2	STEP	0.0
Cleaning	2800	2	STEP	1.0

Statistical analysis

All samples were digested and analysed in triplicate, and the results were expressed as the average of triplicate measurements. GFAAS combined with the ultrasound-assisted digestion was applied for the determination of the V_T and V_{org} in sea cucumbers. Vanadium contents were determined on a dry weight basis. The organic degree was estimated as the ratio of V_T/V_{org}. In all cases, mean values with standard deviations ($n = 3$) are shown. SPSS 13.0 software was used for statistical analysis. Possible correlations between vanadium concentrations and sampling sites, seawater environment and sediment were evaluated with a least squares linear regression and a Pearson correlation analysis. One-way ANOVA and Duncan's multiple comparison test were utilized to compare the data according to species at a significance level of 0.05.

Results and discussion

Tissues distribution

Vanadium concentrations in the tissues of *A. japonicus* cultured in Dalian City are reported in Table 2. Variations in V_{org} and V_T concentrations were observed in blood, intestine, mouth, gonad, body wall, respiratory tree and retractor muscles of *A. japonicus* ($p < 0.05$). The general trend in V_T concentrations among different tissues was blood > intestine > mouth > gonad > body wall > respiratory tree > retractor muscles, and

Table 2 Determination of vanadium contents in different tissues from sea cucumbers *Apostichopus japonicus* of Dalian City

Tissues	V_T (μg/g) (d.w.)	V_{org} (μg/g) (d.w.)	Organic degree (%)
Mouth	1.69 ± 0.07[b]	0.64 ± 0.07[a]	37.9[a]
Retractor muscles	0.56 ± 0.03[a]	UD	–
Intestine	4.92 ± 0.06[c]	1.69 ± 0.07[c]	34.3[a]
Gonad	1.54 ± 0.05[b]	1.01 ± 0.01[b]	77.4[b]
Blood	7.68 ± 0.12[d]	5.32 ± 0.08[d]	69.3[b]
Body wall	1.45 ± 0.04[b]	1.09 ± 0.06[b]	75.2[b]
Respiratory tree	0.16 ± 0.01[a]	UD	–

UD undetected

Values in columns with the same superscripts are not significantly different ($p > 0.05$)

the general trend in V_{org} concentrations among different tissues was blood > intestine > body wall > gonad > mouth. Average V_T in blood (7.68 ± 0.12 μg g^{-1}) and intestinal (4.92 ± 0.06 μg g^{-1}) tissues were considerably higher than in the other ones. Blood displayed the highest V_T and V_{org} concentrations, reaching 7.68 and 5.32 μg g^{-1}, respectively. These findings are similar to the results found within the present research (Ueki et al. 2003). It has been shown that vanadium can bind to plasma proteins; therefore, the vanadium-binding proteins can play a role in the transport of vanadium in blood (Soares et al. 2007). In fact, transferrin and albumin have been reported to be binding proteins for vanadium in human plasma (Chasteen 1983). Mouth and intestine generally contain less organic vanadium (37.9; 34.3 %), while most vanadium with high organic degree was found in blood, body wall and gonad (69.3; 75.2; 77.4 %).

Inter-species-specific differences

The results obtained for fourteen species of edible sea cucumbers from two locations (southern Gulf of California, Xisha Waters of the South China Sea) are shown in Table 3. Levels measured in various sea cucumbers were in the range of 0.18–2.56 μg g^{-1} for V_T, which is consistent with observations reported in previous studies (Liu et al. 2012), and 0.16–2.19 μg g^{-1} for V_{org}. Average V_T levels of 1.09 and 0.79 μg g^{-1} for V_{org} were found in edible sea cucumbers. Thus, it was shown that most vanadium in the sea cucumbers exists in organic form and the percentage of organic vanadium in all sea cucumber species ranged from 60.4 to 88.9 %. Several general trends can be highlighted for V_T in the body wall of sea cucumbers from Waters of the South China Sea, which levels were highest in Holothuriidae family (*Holothuria scabra* particularly, 2.56 ± 0.11 μg g^{-1}) and lowest in Psychopotidate family (*Psychropotes longicauda*, 0.18 ± 0.01 μg g^{-1}) and Caudinidae family (*Acaudina molpadioides*, 0.55 ± 0.02 μg g^{-1}). V_{org} contents also shared common patterns, with the Holothuriidae family (2.19 ± 0.07 μg g^{-1}) being the most impregnated and Psychopotidate family (0.16 ± 0.05 μg g^{-1}) the least. Multiple comparison method was also applied to determine which sea cucumber species are significantly different from others. V_{org} and V_T concentrations varied significantly ($p < 0.001$) in the body wall of all samples from Xisha Waters of the South China Sea, while there were also significant differences ($p < 0.001$) in the body wall of all samples from the southern Gulf of California. It is interesting to note that the levels of total vanadium and organic vanadium in *H. scabra* were approximately fourteen times higher than those in *P. longicauda*, but they contain identical percentages of organic vanadium (*H. scabra*, 85.5 %; *P. longicauda*, 88.9 %). Because they contained the highest concentration of vanadium (2.56 ± 0.11 μg g^{-1}), as well as a higher percentage of organic vanadium (85.5 %) compared with all other samples, *H. scabra* samples may employ a more effective accumulation mechanism. Variations in the metal levels of invertebrates might be related to a number of factors such as species, feeding habits, bioavailability of chemicals in food and water and physio-chemical parameters of the aquatic environment, which are reported in previous studies (Dusek et al. 2005; Mason et al. 2000; Joiris et al. 1997; Anan et al. 2005). Thus, besides species-specific factors, variations in the species–concentrations relationship may also be partially attributed to the influence of

Table 3 Determination of vanadium levels and percentage of organic vanadium in different species of sea cucumbers

Family	Species	*n*	V_T (μg g^{-1})	V_{org} (μg g^{-1})	Organic degree (%)
Xisha Waters (South China Sea)					
Stichopodidae	*Stichopus variegatus*	32	0.75 ± 0.03	0.65 ± 0.03	66.0
	Thelenota ananas	14	0.74 ± 0.02	0.39 ± 0.03	60.4
Holothuriidae	*Pearsonothuria graeffei*	17	0.97 ± 0.03	0.73 ± 0.02	75.3
	Holothuria scabra	10	2.56 ± 0.11	2.19 ± 0.07	85.5
	Holothuria fuscogliva	21	1.50 ± 0.03	1.13 ± 0.03	75.3
	Actinopyga mauritiana	15	1.37 ± 0.05	1.09 ± 0.05	79.6
	Bohadschia marmorata	23	1.67 ± 0.09	1.46 ± 0.06	87.4
Psychopotidate	*Psychropotes longicauda*	17	0.18 ± 0.01	0.16 ± 0.05	88.9
Caudinidae	*Acaudina molpadioides*	18	0.55 ± 0.02	0.42 ± 0.06	76.4
Southern Gulf of California					
Stichopodidae	*Isostichopus fuscus*	18	1.33 ± 0.05	0.81 ± 0.03	75.2
	Isostichopus badionotus	23	1.09 ± 0.03	1.01 ± 0.03	77.4
	Parastichopus californicus	21	1.00 ± 0.02	0.82 ± 0.02	65.6
Holothuriidae	*Holothuria mexicana*	14	0.92 ± 0.05	0.69 ± 0.03	75.0
Cucumariidae	*Cucumaria frondosa*	14	0.70 ± 0.03	0.45 ± 0.02	64.3

biological factors (i.e. metabolism), bioavailability of pollutants, environmental and geographical characteristics, and we need more research to explore this issue.

Seasonal variations

Harvesting of sea cucumbers is conducted in two seasons, spring (April–May) and fall (October–November). The sea cucumbers grew roughly from April to early June and grew again from October to December after aestivation from July to September, and then, they went into hibernation from January to March. During the present study, a total of 33 sea cucumber (*Apostichopus japonicus*) samples were collected, 16 in May and 17 in November, from the coast of Bohai Bay and Yellow Sea in eastern china (May and November) to have a better understanding of the seasonal effects on vanadium accumulation. Our data (shown in Table 4) noted that the total vanadium concentrations in edible sea cucumbers vary significantly with seasonality. For *Apostichopus japonicus* harvested from the coast of Bohai Bay, V_T in May were 40.0 % higher (2.15 ± 0.13 μg g^{-1}) than in November (1.45 ± 0.04 μg g^{-1}), which showed that vanadium achieved comparatively higher mean concentrations in May, while lower concentrations were recorded in November; thus, the vanadium levels in *A. japonicus* have marked seasonal variations. Similarly, marked seasonal fluctuations of vanadium levels were also observed in *A. japonicus* harvested from the coast of Yellow Sea. For *A. japonicus* harvested from the coast of Yellow Sea, V_T in May was 48.3 % higher (1.53 ± 0.04 μg g^{-1}) than in November (1.06 ± 0.03 μg g^{-1}). *A. japonicus* is a cold water species, its optimal temperature range being 20–25 °C, and the optimum salinity range for *A. japonicus* is 27–35 ppt. Salinity and temperature are two of the most important abiotic factors affecting the growth and survival of aquatic organisms (Hu et al. 2010). Considering that higher temperature (15.3–21.2, 16.2–21.8 °C) and lower salinities (27.79, 28.25 ppt) were found from the coast of Bohai Bay and Yellow Sea in May than that in November (13.2–18.1 °C, 31.76 ppt; 12.7–20.2 °C, 31.15 ppt), the seasonal changes to vanadium levels could be ascribed to both environmental and biological factors, including the effects of temperature and salinity, which have an important role in transferring metals from seawater to cells which have the capacity for vanadium accumulation. Temperature is a controlling factor of an aquatic organism's activity, while salinity is an indirect factor that modifies numerous physiological responses, such as metabolism, growth and nutrition as well as inter-species-specific

Table 4 Determination of vanadium contents in different sampling seasons from sea cucumbers *Apostichopus japonicus*

Seawater environment	City	*n*	Sampling season	Temperature (°C)	Salinity (ppt)	V_T (μg g^{-1})
Bohai Bay	Dalian	16	May	15.3–21.2	27.79	2.15 ± 0.13
		17	Nov	13.2–18.1	31.76	1.45 ± 0.04
Yellow Sea	Weihai	16	May	16.2–21.8	28.25	1.53 ± 0.04
		17	Nov	12.7–20.2	31.15	1.06 ± 0.03

relationships. Variations in salinity and temperature may change water quality parameters, such as dissolved oxygen and CO_2 levels, NH_3 to NH_4^+ ratio and pH, and the osmotic pressure of the coelomic fluid changes with changing ambient water parameters (Dong et al. 2008). In the present study, vanadium accumulation in sea cucumbers exhibited a unique seasonal pattern with the highest values observed during the monsoon season (May) and lowest during non-monsoon mouths (November). This variation may be attributed to huge run-off from the adjacent landmasses during the monsoon, which results in the decrease of salinity. The lowering of pH might facilitate the dissolution of the precipitated form of metals and increase the amount of metallic ions in solutions. Furthermore, the phytoplankton levels were lower in winter than in spring due to weak wind and ice covering. Therefore, establishing a basic level of knowledge concerning the vanadium changes of *A. japonicus* at different temperatures and salinities is important in the study of accumulation mechanisms and possible physiological roles of vanadium in sea cucumbers.

Locational differences

Table 5 summarizes vanadium levels obtained in sea cucumber *A. japonicus*, sediment and seawater from different sampling locations in coastal areas of China. The study area is located between latitude 27°1′ and 41°1′ north, coastal sea habitats of East Asia, which consist of Bohai Bay, Yellow Sea and East China Sea. The area shown in Fig. 1 was represented by eight sampling sites in China where the sea cucumbers *A. japonicus* were caught in November of 2012. The vanadium levels of the sea cucumbers *A. japonicus* from different sampling locations in coastal areas of China were in the range of 0.28–1.45 μg g^{-1} for V_T and 0.17–1.09 μg g^{-1} for V_{org}, and the percentages of organic vanadium were in the range of 60.4–77.4 %. Among available data, total vanadium levels in the sea cucumbers *A. japonicus* collected at the sampling sites (Dalian, 1.45 ± 0.04 μg g^{-1}; Penglai, 1.33 ± 0.05 μg g^{-1}) along the Bohai Bay and sites (Weihai, 1.06 ± 0.03 μg g^{-1}; Qingdao, 1.11 ± 0.09 μg g^{-1}) along the Yellow Sea were above those collected along the East China Sea. The lowest V_T concentration is observed in sites along the East China Sea (Wenzhou, 0.28 ± 0.02 μg g^{-1}). A high average organic vanadium percentage of 67.0 % was obtained. The highest values for organic vanadium percentage were observed mainly in the area of the Bohai Bay, including Jinzhou (70.1 %), Dalian (75.2 %) and Penglai (77.4 %), whereas average or below-average values were found in the rest of sites. With the increase in latitude, higher organic vanadium percentages were found in the samples in middle latitudes, especially those samples collected from sampling sites along the coastline of the Bohai Bay (Dalian City, Penglai City). Particulate organic matter after sedimentation plays an important role in the material and energy transfer from primary producers to benthic consumers in aquatic ecosystems, and it is a potentially available food source for sea cucumbers (Ren et al. 2010). The present study showed that in sea cucumber culture sites, the vanadium levels in sediment were high, which ranged from 84.2 to 125.0 μg g^{-1}. There was a significant correlation between the vanadium levels in sediment and in the sea cucumber ($r = 77.98$ %, $p = 0.00$). In the analysis, significant positive correlations between vanadium in the seawater and V_{org} in the sea cucumber ($r = 81.67$ %, $p = 0.00$), vanadium in the sediment and V_{org} in the sea cucumber ($r = 77.98$ %, $p = 0.00$) were observed, possibly reflecting the geological origin and bioaccumulation mechanism by sea cucumbers. In addition, trace elements in the seawater may be bioaccumulated by sea cucumbers because the sea cucumber needs to filter out oxygen from seawater to breathe. From the

Table 5 The contents and organic degree of vanadium in different sampling sites from sea cucumbers *Apostichopus japonicus*, sediment, seawater

Sampling sites				Sea cucumber			Sediment	Seawater
Seawater environment	Province	City	*n*	V_T (μg g^{-1})	V_{org} (μg g^{-1})	Organic degree (%)	*V* (μg g^{-1})	*V* (μg L^{-1})
Bohai Bay	Liaoning	Jinzhou	17	0.87 ± 0.08	0.61 ± 0.03	70.1	84.2	2.64
	Liaoning	Dalian	24	1.45 ± 0.04	1.09 ± 0.06	75.2	NG	NG
	Shandong	Penglai	15	1.33 ± 0.05	1.03 ± 0.02	77.4	112.8	3.03
Yellow Sea	Shandong	Muping	14	0.90 ± 0.04	0.59 ± 0.02	65.6	98.2	2.54
	Shandong	Weihai	17	1.06 ± 0.03	0.70 ± 0.04	66.0	107.1	2.58
	Shandong	Qingdao	19	1.11 ± 0.09	0.67 ± 0.05	60.4	125.0	2.72
East China sea	Zhejiang	Wenzhou	23	0.28 ± 0.02	0.17 ± 0.02	60.7	NG	NG
	Fujian	Xiapu	25	0.51 ± 0.03	0.31 ± 0.01	60.8	86.3	1.74

NG not given

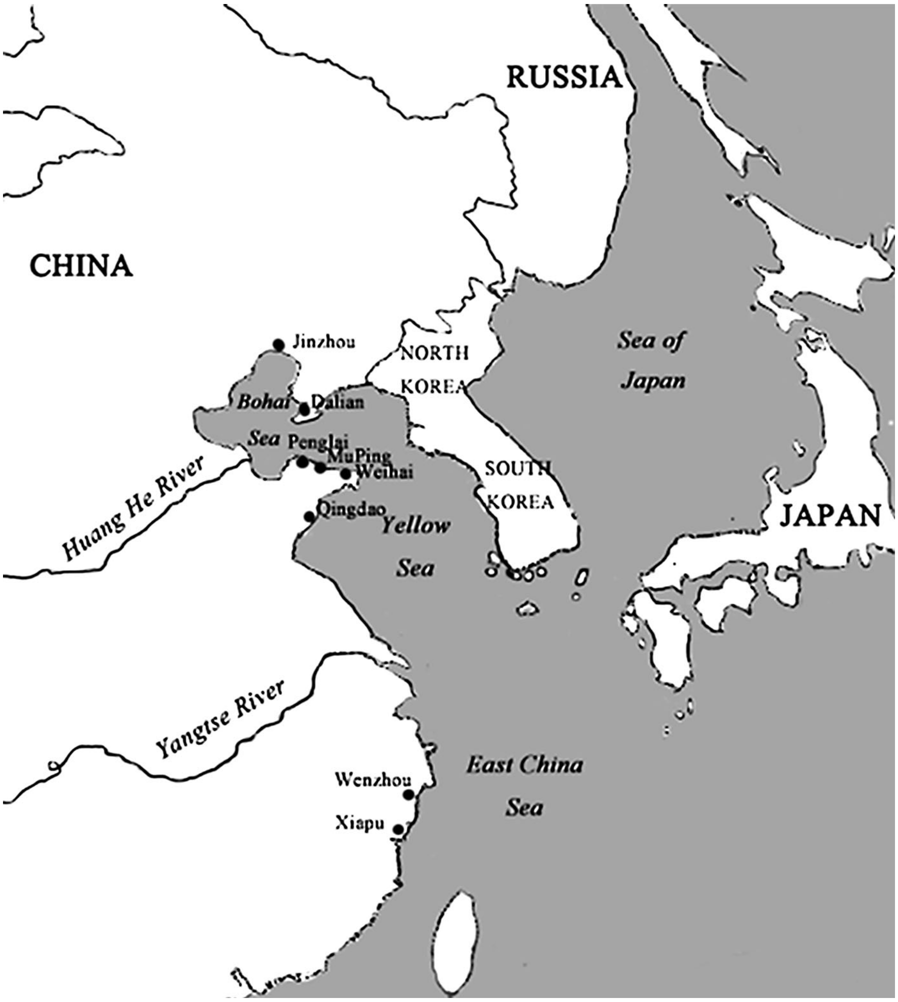

Fig. 1 Map of eight sampling sites in China

results of correlation analysis (bivariate correlations with Pearson's correlation coefficients) among these vanadium concentrations, we found that (1) organic vanadium in sea cucumbers was positively associated with vanadium levels in sediment; (2) organic vanadium in sea cucumbers was positively associated with vanadium levels in seawater.

Overall this study found (1) variations in V_{org} and V_T occurred in blood, intestine, mouth, gonad, body wall, respiratory tree and retractor muscles of *A. japonicus* ($p < 0.05$), (2) inter-species-specific differences in vanadium levels, with *H. scabra* species having the highest levels of vanadium, (3) significant seasonal variations in vanadium levels for sea cucumbers *A. japonicus*, (4) significant correlation between the vanadium levels in sediment and V_{org}, (5) significant correlation between the vanadium levels in seawater and V_{org} and (6) the percentages of organic vanadium in all sea cucumber species ranged from 60.4 to 88.9 %.

Despite the environmental relevance of vanadium in aquatic systems, the bioaccumulation dynamics of this metal have been not intensively investigated (Michibata 2012). As Warnau et al. have reported, metal concentrations in the sea cucumber *Holothuria tubulosa* were associated with the body compartment factor, then to the seasonal one, and finally to the geographical and bathymetric factors (Warnau et al. 2006). Similarly, marked seasonal fluctuations of vanadium levels (approximately 0.5–7.5 $\mu g\ g^{-1}$) were also observed in tissues of the Mediterranean mussel *Mytilusgallo provincialis* from the Adriatic Sea (Fattorini et al. 2008). The available data for vanadium levels in fish species have been reported: approximately 40 different fish species from Qatar and the Caspian Sea exhibited values not exceeding 0.1 $\mu g\ g^{-1}$ in the muscle fillets with a median value of approximately 0.05 $\mu g\ g^{-1}$ (Moati and MAR 1997; Anan et al. 2005). In general, these results seem to suggest that specific regional characteristics can modulate vanadium bioavailability in fish tissue (Fattorini and Regoli 2012). Our results suggest that most vanadium in the sea cucumbers exists in organic form, and these vanadium compounds are present in trace amounts. Our results also suggest that vanadium concentrations in the sea cucumbers studied depend on the seasons (salinity and temperature), species, sampling sites and seawater environment (vanadium contents in seawater and sediment). Bioaccumulation of vanadium in biota can be affected by vanadium concentrations of seawater as well as environmental and water chemistry parameters such as the salinity, temperature and pH values of the seawater. Similar results were also found in the bioaccumulation of trace metals in fish (Yang et al. 2014). Such findings may be due to geological and hydrogeological features surrounding the sampling sites or the accumulation mechanism of sea cucumbers. However, our actual knowledge is still too limited to clarify the mechanism of vanadium variability related to seasons, species-specific and biological factors, and environmental and geographical characteristics. Our investigated taxonomic groups are represented by a small sample size and a more extended database would be necessary to characterize biological and environmental factors affecting vanadium bioavailability and accumulation in aquatic organisms. Given the adverse toxicological effects of inorganic vanadium and positive roles in controlling the development of diabetes on humans, a regular monitoring programme of vanadium contamination in edible sea cucumbers can be recommended.

There is scientific consensus regarding the putative biochemical role for vanadium compounds in living organisms (Michibata 2011). Proteins have been shown to bind vanadium accumulated from seawaters with that reported for ascidians by Michibata et al. where this element is concentrated mainly within vacuoles of the signet ring cells of the so-called vanadocytes (Michibata et al. 1990, 2002). The accumulation and storage of vanadium in ascidians is modulated by a group of vanadium-associated proteins (vanabins) (Kanda et al. 1997). The accumulation mechanism and possible physiological roles of vanadium in sea cucumbers needs further studies, and the biological role of these vanadium compounds is still a rather intriguing subject, and the study of vanadium compounds in sea cucumbers may lead to a new hypoglycaemic material.

Conclusions

We aimed to elucidate the levels, geographical differences, tissues distribution, inter-species-specific and seasonal variations of vanadium compounds in sea cucumbers, as well as several potential factors controlling their distribution in biota. We have observed and reported, for the first time, that the concentration and form of vanadium in sea cucumbers depends on seasons (salinity and temperature), species, sampling sites and seawater environment (vanadium contents in seawater and sediment), and most vanadium exists in organic form in the sea cucumbers and high concentrations of organic vanadium was detected in sea cucumbers. Variations in V_{org} and V_T occurred in blood, intestine, mouth, gonad, body wall, respiratory tree, and retractor muscles of *A. japonicus* ($p < 0.05$) and vanadium concentrations in the blood of the sea cucumber were considerably higher than in the other studied tissues. The knowledge of vanadium concentrations in sea cucumber species is important with respect to human consumption of sea cucumbers. The present study is important not only from the human health point of view but it also presents a comparative account of vanadium in edible sea cucumbers from different seasons, sampling sites and species. Because of the potential positive roles in controlling the development of diabetes on humans, a regular monitoring programme of vanadium contamination in edible sea cucumbers can be recommended. An initial understanding of the levels and biological forms of vanadium in sea cucumbers was established. Scientific exploration must continue to elucidate the mechanism of action of organic vanadium in edible sea cucumbers, which, in turn, will define the contribution of vanadium in human benefit.

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References

Anan, Y., Kunito, T., Tanabe, S., Mitrofanov, I., & Aubrey, D. G. (2005). Trace element accumulation in fishes collected from coastal waters of the Caspian Sea. *Marine Pollution Bulletin, 51*(8–12), 882–888.

Barceloux, D. G., & Barceloux, D. (1999). Vanadium. *Clinical Toxicology, 37*(2), 265–278.

Bellante, A., Sprovieri, M., Buscaino, G., Manta, D. S., Buffa, G., Di Stefano, V., et al. (2009). Trace elements and vanadium in tissues and organs of five species of cetaceans from Italian coasts. *Chemistry and Ecology, 25*(5), 311–323.

Brichard, S., Assimacopoulos-Jeannet, F., & Jeanrenaud, B. (1992). Vanadate treatment markedly increases glucose utilization in muscle of insulin-resistant fa/fa rats without modifying glucose transporter expression. *Endocrinology, 131*(1), 311–317.

Burba, P., & Willmer, P. (1986). Trace determination of molybdenum and vanadium in natural waters by means of atomic spectroscopy (AAS, ICP-OES) after preconcentration. *Fresenius' Zeitschrift für analytische Chemie, 324*(3–4), 298–299.

Chasteen, N. D. (1983). The biochemistry of vanadium. In M. J. Clarke (Ed.), *Copper, molybdenum, and vanadium in biological systems* (pp. 105–138). Berlin: Springer.

Chen, J. (2004). Present status and prospects of sea cucumber industry in China. In A. Lovatelli (Ed.), *Advances in sea cucumber aquaculture and management* (pp. 25–38). Rome: FAO Fisheries technical paper.

Clague, M. J., Keder, N. L., & Butler, A. (1993). Biomimics of vanadium bromoperoxidase: Vanadium (V)–Schiff base catalyzed oxidation of bromide by hydrogen peroxide. *Inorganic Chemistry, 32*(22), 4754–4761.

Clark, T. A., Deniset, J. F., Heyliger, C. E., & Pierce, G. N. (2014). Alternative therapies for diabetes and its cardiac complications: Role of vanadium. *Heart Failure Reviews, 19*(1), 123–132.

Dong, Y. W., Dong, S. L., & Meng, X. L. (2008). Effects of thermal and osmotic stress on growth, osmoregulation and Hsp70 in sea cucumber (*Apostichopus japonicus Selenka*). *Aquaculture, 276*(1–4), 179–186.

Dusek, L., Svobodová, Z., Janousková, D., Vykusová, B., Jarkovský, J., Šmíd, R., et al. (2005). Bioaccumulation of mercury in muscle tissue of fish in the Elbe River (Czech Republic): Multispecies monitoring study 1991–1996. *Ecotoxicology and Environmental Safety, 61*(2), 256–267.

Erdem, A., Shahwan, T., Çağır, A., & Eroğlu, A. E. (2011). Synthesis of aminopropyl triethoxysilane-functionalized silica and its application in speciation studies of vanadium (IV) and vanadium (V). *Chemical Engineering Journal, 174*(1), 76–85.

Evangelou, A. M. (2002). Vanadium in cancer treatment. *Critical reviews in oncology/hematology, 42*(3), 249–265.

Fattorini, D., Notti, A., Di Mento, R., Cicero, A. M., Gabellini, M., Russo, A., et al. (2008). Seasonal, spatial and inter-annual variations of trace metals in mussels from the Adriatic Sea: A regional gradient for arsenic and implications for monitoring the impact of off-shore activities. *Chemosphere, 72*(10), 1524–1533.

Fattorini, D., & Regoli, F. (2012). Hyper-accumulation of vanadium in polychaetes. In H. Michibata (Ed.), *Vanadium: Biochemical and molecular biological approaches* (pp. 73–92). New York: Springer.

Ferreira, S. L. C., Queiroz, A. S., Fernandes, M. S., & dos Santos, H. C. (2002). Application of factorial designs and Doehlert matrix in optimization of experimental variables associated with the preconcentration and determination of vanadium and copper in seawater by inductively coupled plasma optical emission spectrometry. *Spectrochimica Acta, Part B: Atomic Spectroscopy, 57*(12), 1939–1950.

Filik, H., & Aksu, D. (2011). Determination of vanadium in food samples by cloud point extraction and graphite furnace atomic absorption spectroscopy. *Food Analytical Methods, 5*(3), 359–365.

Gil, J., Miralpeix, M., Carreras, J., & Bartrons, R. (1988). Insulin-like effects of vanadate on glucokinase activity and fructose 2, 6-bisphosphate levels in the liver of diabetic rats. *Journal of Biological Chemistry, 263*(4), 1868–1871.

Henze, M. (1911). Untersuchungen über das Blut der Ascidien. I. Mitteilung. Die Vanadiumverbindung der Blutkörperchen. *Hoppe-Seyleŕ s Zeitschrift für physiologische Chemie, 72*(5–6), 494–501.

Heyliger, C. E., Tahiliani, A. G., & McNeill, J. H. (1985). Effect of vanadate on elevated blood glucose and depressed cardiac performance of diabetic rats. *Science, 227*(4693), 1474–1477.

Hu, M., Li, Q., & Li, L. (2010). Effect of salinity and temperature on salinity tolerance of the sea cucumber *Apostichopus japonicus*. *Fisheries Science, 76*(2), 267–273.

Jennings, J. R., & Rainbow, P. S. (1979). Studies on the uptake of cadmium by the crab *Carcinus maenas* in the laboratory. I. Accumulation from seawater and a food source. *Marine Biology, 50*(2), 131–139.

Joiris, C. R., Moatemri, N. L., & Holsbeek, L. (1997). Mercury and polychlorinated biphenyls in zooplankton and shrimp from the Barents Sea and the Spitsbergen area. *Bulletin of Environment Contamination and Toxicology, 59*(3), 472–478.

Kanda, T., Nose, Y., Wuchiyama, J., Uyama, T., Moriyama, Y., & Michibata, H. (1997). Identification of a vanadium-associated protein from the vanadium-rich ascidian. *Ascidia sydneiensis samea. Zoological science, 14*(1), 37–42.

Kawakami, N., Ueki, T., Amata, Y., Kanamori, K., Matsuo, K., Gekko, K., et al. (2009). A novel vanadium reductase, Vanabin2, forms a possible cascade involved in electron transfer. *Biochimica et Biophysica Acta, 1794*(4), 674–679.

Kobayashi, M., & Olefsky, J. M. (1979). Effects of streptozotocin-induced diabetes on insulin binding, glucose transport, and intracellular glucose metabolism in isolated rat adipocytes. *Diabetes, 28*(2), 87–95.

Liu, X., Xue, C., Wang, Y., Li, Z., Xue, Y., & Xu, J. (2012). The classification of sea cucumber (*Apostichopus japonicus*) according to region of origin using multi-element analysis and pattern recognition techniques. *Food Control, 23*(2), 522–527.

Mason, R. P., Laporte, J., & Andres, S. (2000). Factors controlling the bioaccumulation of mercury, methylmercury, arsenic, selenium, and cadmium by freshwater invertebrates and fish. *Archives of Environmental Contamination and Toxicology, 38*(3), 283–297.

Michibata, H. (2011). Vanadium effects on bone metabolism. In H. Michibata (Ed.), *Vanadium: Biochemical and molecular biological approaches* (pp. 145–162). New York: Springer.

Michibata, H. (2012). Hyper-accumulation of vanadium in polychaetes. In H. Michibata (Ed.), *Vanadium: Biochemical and molecular biological approaches* (pp. 73–89). New York: Springer.

Michibata, H., Hirose, H., Sugiyama, K., Ookubo, Y., & Kanamori, K. (1990). Extraction of a vanadium-binding substance (vanadobin) from the blood cells of several ascidian species. *The Biological Bulletin, 179*(1), 140–147.

Michibata, H., Uyama, T., Ueki, T., & Kanamori, K. (2002). Vanadocytes, cells hold the key to resolving the highly selective accumulation and reduction of vanadium in ascidians. *Microscopy Research and Technique, 56*(6), 421–434.

Miramand, P., & Guary, J. C. (1980). High concentrations of some heavy metals in tissues of the Mediterranean octopus. *Bulletin of Environment Contamination and Toxicology, 24*(5), 783–788.

Moati, A., & MAR. (1997). Bio accumulation of chromium, nickel, lead and vanadium in some commercial fish and prawn from qatari waters. *Qatar University Science Journal, 17*(1), 195–203.

Pekiner, O. Z., Naeemullah, & Tüzen, M. (2014). Preconcentration and speciation of vanadium by three phases liquid–liquid microextraction prior to electrothermal atomic absorption spectrometry. *Journal of Industrial and Engineering Chemistry, 20*(4), 1825–1829.

Pillai, S. I., Subramanian, S. P., & Kandaswamy, M. (2013). A novel insulin mimetic vanadium flavonol complex: Synthesis, characterization and in vivo evaluation in STZ-induced rats. *European Journal of Medicinal Chemistry, 63*, 109–117.

Ramachandran, B., Kandaswamy, M., Narayanan, V., & Subramanian, S. (2003). Insulin mimetic effects of macrocyclic binuclear oxovanadium complexes on streptozotocin-induced experimental diabetes in rats. *Diabetes, Obesity and Metabolism, 5*(6), 455–461.

Ramachandran, B., Ravi, K., Narayanan, V., Kandaswamy, M., & Subramanian, S. (2004a). Effect of macrocyclic binuclear oxovanadium complex on tissue defense system in streptozotocin-induced diabetic rats. *Clinica Chimica Acta, 345*(1), 141–150.

Ramachandran, B., Ravi, K., Narayanan, V., Kandaswamy, M., & Subramanian, S. (2004b). Protective effect of macrocyclic binuclear oxovanadium complex on oxidative stress in pancreas of streptozotocin induced diabetic rats. *Chemico-Biological Interactions, 149*(1), 9–21.

Ren, Y., Dong, S., Wang, F., Gao, Q., Tian, X., & Liu, F. (2010). Sedimentation and sediment characteristics in sea cucumber *Apostichopus japonicus* (Selenka) culture ponds. *Aquaculture Research, 42*(1), 14–21.

Sakurai, H., Sano, H., Takino, T., & Yasui, H. (2000). An orally active antidiabetic vanadyl complex, bis (1-oxy-2-pyridinethiolato) oxovanadium (IV), with VO (S_2O_2) coordination mode; in vitro and in vivo evaluations in rats. *Journal of Inorganic Biochemistry, 80*(1), 99–105.

Soares, S. S., Martins, H., Duarte, R. O., Moura, J. J. G., Coucelo, J., Gutiérrez-Merino, C., et al. (2007). Vanadium distribution, lipid peroxidation and oxidative stress markers upon decavanadate in vivo administration. *Journal of Inorganic Biochemistry, 101*(1), 80–88.

Srivastava, A., & Mehdi, M. (2005). Insulino-mimetic and antidiabetic effects of vanadium compounds. *Diabetic Medicine, 22*(1), 2–13.

Takashi, H., Nobuhiro, Z., Kyoko, Y., Ryoko, N., Xue, C., & Tatsuya, S. (2005). Recent advances in researches on physiologically active substances in holothurians. *Journal of Ocean University of China, 4*(3), 193–197.

Ueki, T., Adachi, T., Kawano, S., Aoshima, M., Yamaguchi, N., Kanamori, K., et al. (2003). Vanadium-binding proteins (vanabins) from a vanadium-rich ascidian *Ascidia sydneiensis samea*. *Biochimica et Biophysica Acta (BBA)—Gene Structure and Expression, 1626*(1-3), 43–50.

Ueki, T., Kawakami, N., Toshishige, M., Matsuo, K., Gekko, K., & Michibata, H. (2009). Characterization of vanadium-binding sites of the vanadium-binding protein Vanabin2 by

site-directed mutagenesis. *Biochimica et Biophysica Acta, 1790*(10), 1327–1333.
Ueki, T., Satake, M., Kamino, K., & Michibata, H. (2008). Sequence variation of Vanabin2-like vanadium-binding proteins in blood cells of the vanadium-accumulating ascidian *Ascidia sydneiensis samea*. *Biochimica et Biophysica Acta, 1780*(7–8), 1010–1015.
Ueki, T., Shintaku, K., Yonekawa, Y., Takatsu, N., Yamada, H., Hamada, T., et al. (2007). Identification of vanabin-interacting protein 1 (VIP1) from blood cells of the vanadium-rich ascidian *Ascidia sydneiensis samea*. *Biochimica et Biophysica Acta, 1770*(6), 951–957.
Wang, Y., Su, W., Zhang, C., Xue, C., Chang, Y., Wu, X., et al. (2012). Protective effect of sea cucumber (*Acaudina molpadioides*) fucoidan against ethanol-induced gastric damage. *Food Chemistry, 133*(4), 1414–1419.
Warnau, M., Dutrieux, S., Ledent, G., Rodriguez y Baena, A. M., & Dúbois, P. (2006). Heavy metals in the sea cucumber *Holothuria tubulosa* (Echinodermata) from the Mediterranean *Posidonia oceanica* ecosystem: Body compartment, seasonal geographical and bathymetric variations. *Environmental Bioindicators, 1*(4), 268–285.
Wen, J., & Hu, C. (2010). Elemental composition of commercial sea cucumbers (holothurians). *Food Additives and Contaminants, 3*(4), 246–252.
World health organization. (1984). Vanadium, environmental health criteria 81. In The International (Ed.), *Programme on chemical safety (IPCS)*. Geneva: WHO.
Yang, R., Zhang, S., & Wang, Z. (2014). Bioaccumulation and regional distribution of trace metals in fish of the Tibetan Plateau. *Environmental Geochemistry and Health, 36*(1), 183–191.