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Preliminary Study on Edible Insect Species *Cybister limbatus* (Fabricius 1775) and Its Heavy Element Contents

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ABSTRACT: The objective of the study was to determine the concentration of heavy elements in edible insect pack and estimate the potential health risks of elements to humans via consumption of the insect pack. Aquatic edible insect Diving Beetle pack, *Cybister limbatus* (Fabricius 1775) (Coleoptera: Dytiscidae) was chosen for the study. Sixteen heavy elements (Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Br, Rb, Sr, Pb) were determined in the edible beetle. Energy Dispersive X-ray Fluorescence (EDXRF) was used to determine the concentration of the elements. Among the sixteen studied elements Ca (0.98 ppm), Ti (0.49 ppm) and V (0.38 ppm) had the highest concentration, whereas Rb (0.05 ppm), Sr (0.04 ppm), As (0.32 ppm), Br (0.06 ppm) and Pb (0.06 ppm) had the lowest concentration. The results compared with US institute of medicine (IOM) panel on micronutrient guidelines, the levels of elements in this study were found safe for consumption. But especially residues of Pb and As, which are thought as the potential hazardous elements, in this edible insect may pose health problem in the future.

Keywords: Edible insects, *Cybister limbatus* (Fabricius 1775), entomophagy, EDXRF, Dytiscidae, heavy element.

Yenilebilir Böcek Türü *Cybister limbatus* (Fabricius 1775) ve Ağır Element Seviyeleri Üzerine Bir Ön Çalışma

ÖZ : Bu çalışmanın amacı yenilebilir böcek paketlerindeki ağır element miktarını değerlendirmek ve bu böcek paketinin tüketimi yoluyla insanlar için sağlık riskini değerlendirmektir. Çalışma için sucul yenilebilir böcek *Cybister limbatus* (Fabricius 1775) (Coleoptera: Dytiscidae) seçilmiştir. Bu türde on altı ağır element (Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Br, Rb, Sr, Pb) tespit edilmiştir. Çalışma için EDXRF (Enerji Dağılımlı X-Işını Floresans) spektrometresi kullanılmıştır. Çalışılan 16 element arasında Ca (0,98 ppm), Ti (0,49 ppm) ve V (0,38 ppm) en yüksek konsantrasyonda, Rb (0,05 ppm), Sr (0,04 ppm), As (0,32 ppm), Br (0,06 ppm) ve Pb (0,06 ppm) ise en düşük konsantrasyondadır. Sonuçlar Birleşmiş Milletler İlaç Enstitüsü mikrobesein esasları hakkındaki panel ile karşılaştırıldığında, bu çalışmadaki değerler tüketim için uygun görülmüştür. Fakat, özellikle potansiyel zararlı elementlerden olduğu düşünülen Pb ve As miktarı gelecekte sağlık problemleri oluşturabilir.

Anahtar Sözcükler: Yenilebilir böcek, *Cybister limbatus* (Fabricius 1775), entomofaji, EDXRF, Dytiscidae, ağır element.

INTRODUCTION

Entomophagy is a term that describes eating insects as a food source. Besides insects play an important role in survival of human kind like to help plant reproduction and waste biodegradation,

using their product in technology and engineering etc., also insects serve as a source of food for human. Contrary to many societies, entomophagy or consumption of insects is part of human nutrition in some countries including China, Thailand, India, Korea, Japan, Mexico, New

Zealand, Australia (Bodenheimer, 1951). Hundreds of insect species have been used as human food in these countries. Some of the popular edible insects are grasshoppers, caterpillars, winged termites, bee, wasp and ant brood, winged ants, cicadas, and a variety of aquatic insects (Raubenheimer and Rothman, 2013).

As a food source, insects are highly nutritious, and nowadays entomophagy is a major entomological research interest with focus on its future prospect for food and feed security. In many country insects are clearly a “food of choice” rather than necessity, but in some poorest of society insects are consumed to combat hunger and malnutrition and scientist recommends to people eat more insects to these societies because edible insects have low-fat, high-protein, mineral and vitamin for people (Adeoye *et al.*, 2014; Anankware *et al.*, 2015).

The members of the Dytiscidae (Predacious diving beetles) under the order Coleoptera represented in most of the freshwater and feed vigorously on almost all invertebrates as well as fish eggs. They are active swimmers and swift divers (Nilson and Holmen, 1995). Dytiscidae is highly diverse and estimated to include about 4,000 species in over 160 genera. The genus *Cybister* is the most well-known groups (Deepa, 2009). *Cybister limbatus* (Fabricius 1775) belongs to Dytiscidae and mostly eaten in China, in Africa (by Bantu people), Japan, Senegal, Sierra, Leone, USA, Mexico, Indonesia, Madagascar, Sri Lanka, Thailand, Korea, Myanmar, Vietnam, India, Cameroon, Congo and Laos (Ramos-Elorduy *et al.*, 2009; Mitsuhashi, 2016). Generally, insects have been collected from nature by hand picking but they can also collect with the aid of sweep net, dead raphia or oil palm (Adeoye *et al.*, 2014). Instead of this, insects are now increasingly sold in local markets or online shopping.

Heavy element pollution has expanded many parts of the world, especially developing countries. These elements are quite reactive and generally toxic even at low concentration. When enter

abiotic environment they bioaccumulate in food chain and affect biota negatively (Gall *et al.*, 2015). Heavy metal accumulation level can vary with species and kind of element. Some species are intolerant to environmental disturbance and they change their habitat or getting extinct while others can tolerate the pollution and continue to living in a polluted or naturally high-metal environment. These hyper-accumulator organisms are using in mostly bioremediation studies (Dixit *et al.*, 2015).

These days, contamination of different kind of food products by heavy elements is becoming an unavoidable problem. Studies showed that long time exposure to lead (Pb) and Arsenic (As) via food or air may affect brain development in children and fetus negatively, in adults affect generally kidney and other organs. The aim of the study is to quantify the accumulation of heavy elements in this edible insect pack and to determine whether these levels pose a human health concern. The results obtained from this study would provide information for background levels of elements in the edible insect Diving Beetle pack.

MATERIALS AND METHODS

In this study heavy element concentration of aquatic edible insect Diving Beetle, *Cybister limbatus* (Coleoptera: Dytiscidae) pack was evaluated. The insect sample measured by Energy Dispersive X-Ray Fluorescence (EDXRF) spectrometry at Atatürk University Professor Dr. H. C Wolf Weyrich High Energy Spectrometer Research Laboratory. Different kind of samples such as liquid, gas, soil or powder can be analyzed easily and wide range of elements can be measured simultaneously with EDXRF. The edible insect pack was taken from online. Only one insect was enough to measure heavy element content with EDXRF and one insect weight was 0.098 g. Firstly, the sample dried in an oven at 80°C for 36 hours to extract moisture then pulverized in mortar and cellulose was added as a binder. Five tons of pressure applied to make 13 mm diameter pellet. 5 Ci ²⁴¹Am radioactive source and an HPGe detector

with resolution ~180 eV at 5.9 keV was used to determine the heavy elements in 13 mm diameter pellet of *Cybister limbatus*. The sample was excited by using 59.5 keV photons which emitted from ^{241}Am radioactive source. The measurement was carried out under vacuum. The concentration of elements in the sample was determined by WinAXIL software.

RESULTS

The current study describes the dietary exposure to heavy elements in consuming the edible insect and heavy element concentration of this edible insect pack was evaluated. Results of measured sixteen elements were given in Table 1, and photo of the studied insect *Cybister limbatus* (Fabricius 1775) were given in Figure 1.



Figure 1. *Cybister limbatus* (Photo by: Zeynep Aydoğan).
Şekil 1. *Cybister limbatus* (Fotoğraf: Zeynep Aydoğan).

Among the sixteen studied elements Ca (0.98 ppm), Ti (0.49 ppm) and V (0.38 ppm) had the highest concentration, whereas Rb (0.05 ppm), Sr (0.04 ppm), As (0.32 ppm), Br (0.06 ppm) and Pb (0.06 ppm) had the lowest concentration.

Bio-accumulation of the elements in *Cybister limbatus* showed a trend in the

Ca>Ti>V>Cr>As>Mn>Co>Ni>Fe>Cu>Zn>Se>Br
=Pb>Rb>Sr.

Table 1. Heavy element concentrations in *Cybister limbatus*.
Çizelge 1. *Cybister limbatus* 'un ağır element konsantrasyonları.

Heavy Element Ağır element	<i>Cybister limbatus</i> (ppm)
Ca	0.98
Ti	0.49
V	0.38
Cr	0.34
Mn	0.30
Fe	0.17
Co	0.25
Ni	0.19
Cu	0.15
Zn	0.13
As	0.32
Se	0.08
Br	0.06
Rb	0.05
Sr	0.04
Pb	0.06

DISCUSSION AND CONCLUSIONS

Insects are eaten traditionally in most cultures and are playing an important role in human nutrition and provide many nutrients to the consumer especially the people who suffer from malnutrition. Eating edible insect at sufficient levels can promote the level of essential elements and also vitamins to human body. Even though insects are not used in Turkish food culture, it is a growing industry as an alternative source of unprocessed raw materials in the world. Potential of insect to bio-accumulate chemical substances it is probable to accumulate in the consumers' body which later may reach toxic concentration. It is therefore necessary to control the levels of these toxic elements in food in order to protect human health. Besides to insects' nutritional value there is no standard that only refer to use of insects as food.

Permissible daily intakes of the measured elements were given in below;

Ca has an essential role in nervous system, muscles, bone and tooth. Recommended daily intake for male/female is 1000 mg/d (Anonymous, 2001). In this study Ca level is found within the normal level (0.98 ppm).

Cr predominantly found in the body as trivalent form (Cr^3) and helps to metabolism the fats and carbohydrates. Recommended daily intakes for male/female are 35 and 25 $\mu\text{g/d}$ respectively (Anonymous, 2001). In this study Cr level is found as 0.34 ppm.

Mn is necessary for the brain and nerve function, required for metabolism and component of some enzyme. Recommended daily intakes for male/female are 2.3 and 1.8 mg/d respectively (Anonymous, 2001). In this study the insect Mn level is found as 0.30 ppm.

Fe is a component of hemoglobin and many enzyme systems. Recommended daily intakes for male/female are 8 and 18 mg/d respectively (Anonymous, 2001). In this study the insect Fe level is found as 0.17 ppm.

Co is a part of cobalamin or vitamin B_{12} and help to produce red blood cell. In the literature recommended daily intake of Co is variable and may be as much as 1 mg. In this study the insect Co level is found as 0.25 ppm.

There has been no clear identified biological function of Ni in human but it can be component of some metabolic enzymes and therefore performs vital functions in metabolism. There is no reference daily intake for Ni in IOM list (Anonymous, 2001) but Anke *et al.* (1984) gave Ni value for human requirements less than 500 $\mu\text{g/kg}$. In this study the insect Ni level is found as 0.19 ppm.

Cu is a cofactor of many redox enzymes IOM recommended daily intake for man and woman is 900 $\mu\text{g/d}$ (Anonymous, 2001), and in this study measured level is 0.15 ppm.

According to Marger *et al.* (2014) divalent form of Zn is the second most abundant element in the human body and modulates the activity of protein folding and function. IOM recommended daily intake for man and woman is 11 and 8 mg/d respectively (Anonymous, 2001), in this study measured level is 0.13 ppm.

Se studies showed that it has possible protective effects against cancer and other chronic disease (Fairweather-Tait *et al.*, 2011). IOM recommended daily intake for man and woman is 55 $\mu\text{g/d}$, in this study measured level is 0.08 ppm (Anonymous, 2001).

There is no clear statement of biological function of Br and it has no toxicological concern in nutrition. In the WHO report (Anonymous, 2009) it is stated that some hemodialysis patients, who has insomnia, is related to Br deficiency. In the same report express that 320 mg/l plasma Br level may sometimes fatal. In this study measured Br level in the insect is 0.06 ppm.

Ti, V, As, Rb, Sr and Pb has no biological function in human body. In this study, levels of these elements in the insect total body are 0.49, 0.38, 0.32, 0.05, 0.04 and 0.06 ppm respectively. Elemental Ti is not reactive but the researchers from UCLA indicated that Ti damages to DNA and chromosome (Trouiller *et al.*, 2009). According to IOM maximum level of daily V intake in both man and woman is 1.8 mg/d and is not likely to pose a risk of adverse effects (Anonymous, 2001). US drinking water standards stated that $>50 \mu\text{g/l}$ As lead to cancer, cardiovascular and neural disease (Anonymous, 1980). According to ATSDR Sr is found everywhere in small degree and can be exposed to low levels via food, breathing and water (Anonymous, 2004). Stable form of Sr is not harmful but strontium chromate is hazardous due to toxic form of chromium not Sr itself. When it enters to body, acts like calcium and accumulate in the bone. This may lead to weakened of growing bone. Radioactive Sr may cause cancer but there is no any data indicate that stable Sr cause cancer.

Residues of Pb, As etc. in this edible insect may pose health problem in the future. Toxicity of As depend on mostly its chemical form i.e. arsenide and its compounds are toxic for human health, its dose and duration of exposure are also important. Exposure or intake inorganic As via food, medications, work places or environment leads to multi-organ and system dysfunction such as skin,

cardiovascular, nervous system, gastrointestinal system (Mazumder, 2008). Like As, Pb level in organisms is a sign of contamination because they are not essential for biota. According to WHO report in the general population source of lead uptake predominantly based on food (Tong *et al.*, 2000). Lead generally stored in bone but this storage is an age dependent process. While lead deposits in spongy bones of children, in adults leads is deposited in spongy cortical bone and teeth. Iron and Calcium supplementation impair lead uptake and decrease the absorption of lead. In literature low level exposure of lead (blood level below 10 µg/dl) cause neurological damage, cognitive disorders and renal dysfunction (Tong *et al.*, 2000; Patrick, 2006). In the present study lead level was measured 0.06 ppm.

As it seen the Table 1 all the heavy elements are far below the tolerable limits for consumption.

However, the whole values of heavy elements are acceptable for human consumption; adequate check should be maintained against the risk of these elements in the *Cybister limbatus* pack. Because we do not ensure that adequate actions were taken of its production, manufacture, preparation and packaging to reduce contamination of insect pack. Although our study did not show any clear relationship between heavy element concentration in the selected insect and its environment, our data still suggest that consuming this insect should be monitored for heavy element level. This contamination may be via naturally or anthropogenically. More comprehensive study should be done to clarify such comparison. Industrialized edible insects may include less heavy element than the collected from nature. The study needs to more research to understand if industrialized edible insects more safe for consumption than the collect from nature.

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