Heavy Metal Accumulation in Muscle Tissue of *Microtus guentheri* (Danford and Alston 1880) from Korkuteli-Antalya, Turkey

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Abstract

Different sources, including mining activities are polluting the environment by heavy metal release. The objective of this study was to determine the concentrations of seventeen heavy metals (Fe, Al, Zn, Cu, Cr, Mn, Sr, Ni, Ba, Se, V, Sn, Pb, As, Co, Mo and Cd) in muscle from wild rodent Microtus guentheri from a natural region which is near mine and stone quarry activities in Korkuteli-Antalya. Prior to this study there was no information available on the heavy metal accumulation in muscle tissue for this species in this region. It's important to determine the heavy metal accumulation levels of muscle in Microtus guentheri as biomonitor. The order of mean concentration of the heavy metals in samples was Fe>Al>Zn>Cu>Cr>Mn>Sr>Ni>Ba>Se>V>Sn>Pb>As>Co>Mo>Cd. The toxic heavy metal pollution began in the vicinity of Korkuteli. Case, is thought to be due to mine sources, like marble and stone quarries and maybe agricultural activities.

Key words: Heavy metal accumulation, *Microtus guentheri*, Ecotoxicology, Muscle tissue

1. Introduction

Heavy metal pollution is a growing environmental problem. There are numerous sources of heavy metal pollution, including coal, natural gas, metal, paper industries. (Marcheselli et al., 2010; Blagojević et al., 2012). Heavy metals can persist for a long time in the soil and cause health problems in living organisms (Ashraf et al., 2012*;* Okuku and Peter, 2012). Wild animals living in polluted areas are exposed to various heavy metals because of mining activities (Marques et al., 2007). Sentinel organisms are suitable tools for biomonitoring. Small rodent species inhabiting near mine tailings are very important biomonitor organisms for revealing the effects of heavy metal exposure (Tovar-Sánchez et al., 2012). According to the literature, heavy metal accumulations were observed in the different organs and tissues of small rodents living in polluted areas (Blagojević ve ark., 2012; Martiniaková, 2010; Wijnhoven et al., 2007; Zarrintab & Mirzaei, 2017). Metal concentrations in wild small mammals are often measured in the target organs (Damek-Poprawa and Sawicka-Kapustra 2003, 2004; Wijnhoven et al., 2007; Zarrintab and Mirzaei, 2017). In this study, *Microtus guentheri*, which is inhabited in the Korkuteli-Antalya near mine and stone quarry regions was used as biomonitor species for heavy metal accumulation in muscle tissues. Our findings are presented with the aim to determine the pollution levels in Korkuteli with a bimonitor rodent namely Levant Vole. This is the first preliminary study to determine the accumulation levels of different heavy metals in muscle tissue of *M. guentheri* on this region where polluted by mining activies in Korkuteli.

2. Materials and methods

This research is based on 6 living voles, taken from the different sites in Korkuteli-Antalya, Turkey (in 2016, spring).

2.1. Field studies

Levant Voles were caught in various habitats in six different sites in which trapping were carried out (Fig. 1).

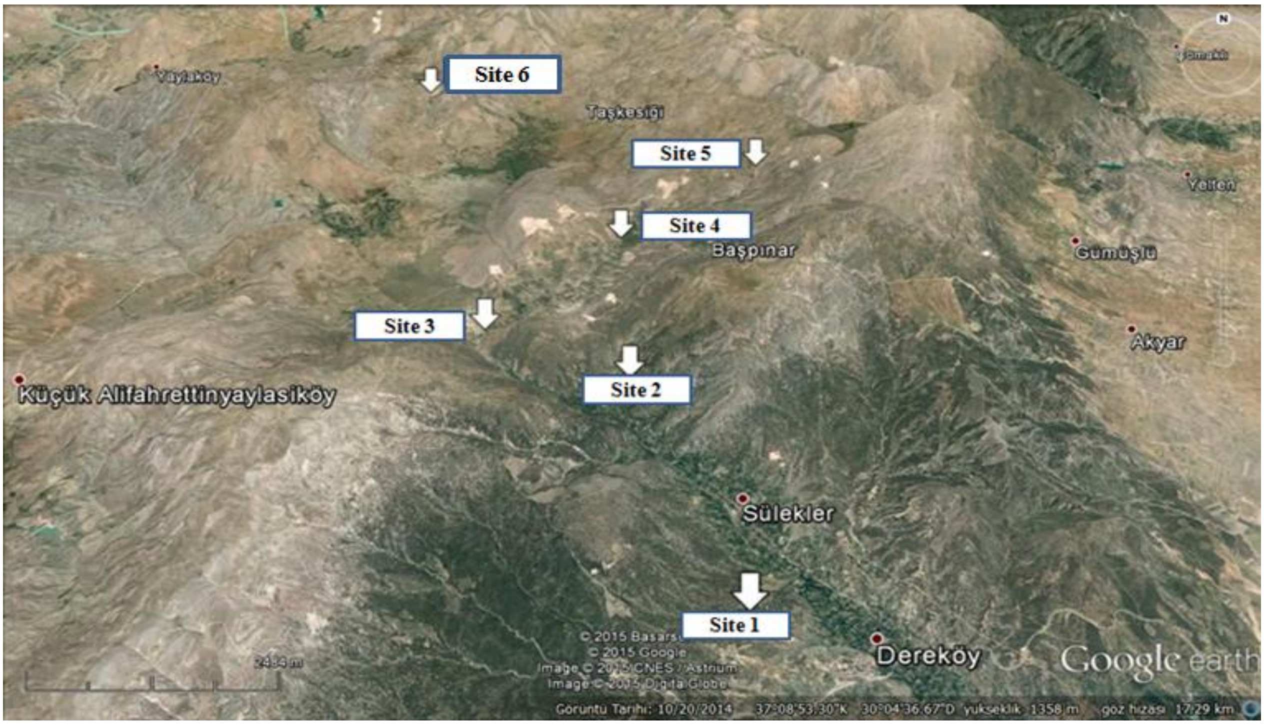

Fig. 1. Map of locations of study sites in Korkuteli-Antalya, Turkey.

We worked with muscle samples of six voles specimens from one season (in 2016, spring) collected by means of Sherman live traps in each site, in Korkuteli-Antalya. The bait used in the traps consisted of roasted peanuts mixed with some chewed bread, according to Yavuz et al., 2017. Captured specimens were killed by cervical dislocation and muscle samples were immediately removed, weighed and frozen at -40 °C prior to chemical analyses in polystyrene tubes. Muscle samples were removed from deep freeze and then allowed to dissolve for a period of time at room temperature. Muscle samples were set at 80-105 °C until completely dry and fixed weight. Also, microwave method was applied for the digestion produce of samples. Samples homogenized by milling prior to analyses. Acid microwave digestion was carried out in a Berghof speedwave MWS-2 microwave. From each tissue, 0.5 g homogenates were placed in a teflon digestion vessel with mix: 8 mL 65% nitric acid (HNO_3) and 2 mL 30% hydrogen peroxide (H_2O_2). After digestion the samples were cooled to room temperature and diluted with ultra-pure water. Then, samples were analyzed by Inductively Coupled Plasma-Mass Spectometer (ICP-MS). For control purposes, the same procedures were carried out for a blind sample on the same conditions. The concentrations of heavy metals were expressed for the muscle samples as milligrams per kilogram (ppm) for dry weight. In this study, the results were given on dry weight (dry wt.) basis. In accordance with this objective for comparative purposes (with the results of other studies), the 29.3% ratio in muscle is used to convert wet weight to dry weight, as (Millán et al., 2008).

2.2. Statistical analysis

From the final data, descriptive statistical characteristics were calculated (mean, standard error) for muscle tissues of *M. guentheri.*

3. RESULTS

The mean concentrations of the metals accumulated in muscle samples of *M. guentheri* as follows (M±Se, n=6): Fe: 268.58±54.14, Al: 226.82±97.45, Zn: 67.60±9.11, Cu: 8.56±0.12, Cr: 7.19±1.43, Mn: 5.97±1.42, Sr: 2.73±0.46, Ni: 1.27±0.49, Ba: 0.96±0.22, Se: 0.54±0.05, V: 0.52±0.17, Sn: 0.46±0.11, Pb: 0.36±0.08, As: 0.23±0.06, Co: 0.21±0.08, Mo: 0.17±0.03, Cd: 0.001±0.0008 ppm. The order of mean concentration of the heavy metals in samples was Fe>Al>Zn>Cu>Cr>Mn>Sr>Ni>Ba>Se>V>Sn>Pb>As>Co>Mo>Cd. According to this data; The toxic heavy metal pollution began at the vicinity of Korkuteli. Case, is thought to be due to mine sources, like marble and stone quarries and maybe agricultural activities.

4. DISCUSSION

Small mammals commonly use as a bioindicator for environmental pollution. Metal accumulation levels in wild small mammals are often measured in different target organs and tissues (muscle, kidney, liver etc.) according to metal types (Blagojević ve ark., 2012; Martiniaková, 2010; Wijnhoven et al., 2007; Zarrintab & Mirzaei, 2017). The mean concentrations of the metals accumulated in muscle samples of *M. guentheri* as follows (M±Se, n=6): Fe: 268.58±54.14, Al: 226.82±97.45, Zn: 67.60±9.11, Cu: 8.56±0.12, Cr: 7.19±1.43, Mn: 5.97±1.42, Sr: 2.73±0.46, Ni: 1.27±0.49, Ba: 0.96±0.22, Se: 0.54±0.05, V: 0.52±0.17, Sn: 0.46±0.11, Pb: 0.36±0.08, As: 0.23±0.06, Co: 0.21±0.08, Mo: 0.17±0.03, Cd: 0.001±0.0008 ppm.

In a previous study, when in muscle samples of *M. guentheri* in Burdur-Çaltepe Turkey, which near marble and stone quarry activities the mean concentrations of heavy metals were found as follows; Fe: 137.14, Al: 72.65, Zn: 24.26, Mn: 3.19, Cu: 1.92, Ni: 1.21, Hg: 0.76, Cr: 0.56, Cd: 0.69,

Pb^{206}: 0.38, Pb^{208}: 0.28, Co: 0.21, B: 0.18, As: 0.12 ppm, in clean area the results were found as follows; Fe: 135.38, Al: 31.52, Zn: 32.31, Mn: 1.21, Cu: 2.13, Ni: 1.11, Hg: 0.66, Cr: 0.55, Cd: 0.48, Co: 0.11, Pb^{206}: 0.25 Pb^{208}: 0.17, B: 0.15, As: 0.13 ppm. For values obtained for Fe, Al, Zn, Cu, Cr, Mn, Ni, Pb, As, Co the accumulation concentration values in muscle tissue are higher than the clean area literature values. Besides in our study, except Co the heavy metal values, namely, Fe, Al, Zn, Cu, Cr, Mn, Ni, Pb, As are even higher than polluted site sample values that were obtained from Burdur-Çaltepe, Turkey (Yavuz & Aktaş, 2017).

Accumulation concentrations of the heavy metal, which are in the samples, were collected from polluted areas in Korkuteli, higher than the literature mean values. For example, the value obtained for Fe, the accumulation concentration value ($\bar{X}$=268.58±54.14 ppm) is significantly higher than the literature values in terms of muscle tissue. The values are; 137.14 ppm for muscle tissue from *M. guentheri* collected from active mine sites in Turkey and 135.38 ppm in a clean area in Turkey (Yavuz & Aktaş, 2017). Besides, for muscle tissue the values are 148.05 ppm from *Peromyscus maniculatus* and 117.65 ppm from *Microtus pennsylvanicus* samples collected from oil sands and surface mines in Canada (Rodriguez-Estival & Smits, 2016) and 187.37 ppm from *Acomys cahirinus* samples collected from polluted areas in Egypt (Mohallal & Younes, 2015). Similarly, accumulation concentrations of Al ($\bar{X}$=268.58±54.14 ppm) in muscle tissue in our study significantly higher than over 3 fold the literature values. The values are; 9.86 ppm for *Peromyscus maniculatus* and 7.00 ppm for *Microtus pennsylvanicus* from oil sands and surface mines in Canada (Rodriguez-Estival & Smits, 2016), 12.02 ppm from muscle of *Peromyscus gossypinus* on different contaminated areas in USA (Reinhart, 2003), 72.65 ppm for *Microtus guentheri* from active mine sites in Turkey and 31.52 ppm from the clean area in Turkey (Yavuz & Aktaş, 2017).

According to our study, results show that Zn accumulation ($\bar{X}$=67.60±9.11) level in muscle tissue was generally higher than the world studies but with two exceptions. The exceptional higher values are; 111.12 ppm for *Meriones persicus* from polluted sites (Okati et al., 2013) and 101.23 ppm for *Heliophobius argenteocinereus* from city's different sites from Malawi (Umbera et al., 2003). Many studies that have lower values than our study value in the literature for muscle tissue of different rodent species. In the literature, Zn accumulation values ranging from 24.26 to 63.76 ppm. These values are; 32.31 ppm for *Microtus guentheri* obtained from relatively clean area and 24.26 ppm for *Microtus guentheri* obtained from near active mine sites in Turkey at the same study (Yavuz & Aktaş, 2017,), 47.82 ppm for *Microtus pennsylvanicus* and 48.29 for *Peromyscus gossypinus* which collected from oil sands and surface mines in Canada (Rodriguez-Estival & Smits, 2016), 40.74 ppm for *Rombomys opimus* and 63.76 for *Rattus norvegicus*, which captured from different land uses with city in Iran (Zarrintab & Mirzaei, 2017), 46.20 ppm for *Clethrionomys glareolus* which captured from near the active mine site in Ireland (Milton & Johnson, 1999), 51.90 ppm for *Clethrionomys glareolus* obtained from an abandoned lead mine site in UK (Milton et al., 2003), 55.97 ppm for *Acomys cahirinus* captured from polluted sites in Egypt (Mohallal &Younes, 2015).

The values obtained from our study correlate ($\bar{X}$=8.56±0.12) with the literature values in terms of accumulation concentration of Cu in muscle tissue: The values are; 6.02 ppm for *Rombomys opimus* and 7.57 ppm for *Rattus norvegicus* from different land uses with city in Iran (Zarrintab & Mirzaei, 2017), 7.99 ppm for *Microtus pennsylvanicus* and 9.66 ppm for *Peromyscus maniculatus* from oil sands and surface mines in Canada (Rodriguez-Estival & Smits, 2016), 8.80 ppm for *Heliophobius argenteocinereus* city's different habitats in Malawi (Umbera et al., 2003), 9.56 ppm for *Acomys cahirinus* from polluted areas in Egypt (Mohallal & Younes, 2015), 4.25 ppm for *Peromyscus gossypinus* from polluted areas in USA (Reinhart 2003), 2.13 ppm for *Microtus guentheri* relatively clean area and 1.92 ppm for near active mine sites in Turkey (Yavuz & Aktaş, 2017). But, unlike these literature studies, a study results indicate that Mn accumulation concentration is 32.40 ppm in muscle tissue of *Meriones persicus* from polluted sites in Iran (Okati et al., 2013). The underlying reasons for these consequences may be characteristic contamination properties of the polluted areas and the variation of the indicator rodent species.

Muscle Ni accumulation level in our study was ($\overline{X}$=1.27±0.49 ppm) similar with a study was carried out in both relatively clean area (1.11 ppm) and active mine site areas (1.21 ppm) in Turkey for same rodent species namely, *M. guentheri* (Yavuz & Aktaş, 2017). But other literature data obtained for Ni accumulation from muscle tissue of different rodent species display variability. Values are: 0.22 ppm for *Peromyscus gossypinus* from polluted areas in USA (Reinhart 2003), 0.48 ppm for *Rattus norvegicus* and 0.78 ppm for *Rombomys opimus* different land uses with city in Iran (Zarrintab & Mirzaei, 2017), 7.95 ppm for *Peromyscus maniculatus* and 10.62 ppm for *Microtus pennsylvanicus* (Rodriguez-Estival & Smits, 2016), 9.56 ppm for *Acomys cahirinus* from polluted areas in Egypt (Mohallal & Younes, 2015), 28.60 ppm for *Meriones persicus* polluted areas in Iran (Okati et al., 2013).

Muscle As accumulation level in the current study was ($\overline{X}$=0.23±0.06 ppm) accordance with literature data. In the world studies mean values of As accumulation in muscle tissues of wild rodent species are; 0.13 ppm for *M. guentheri* from relatively clean area and 0.12 ppm from active mine site areas from Turkey (Yavuz & Aktaş, 2017), 0.75 ppm for *Apodemus sylvaticus* and 0.72 ppm for *Clethrionomys glareolus* from near abandoned mines in UK (Erry et al., 2005).

Muscle Pb accumulation levels in our study were lower than the literature ($\overline{X}$=0.36±0.08). Our results correlate with some literature studies. According to results obtained from Reinhart (2003) and Umbera et al. (2003) show that muscle Pb level lower than our results; for *Peromyscus gossypinus* value 0.13 ppm pollutes areas in USA and for *Heliophobius argenteocineres* value 0.25 city's different habitats in Malawi. For *M. guentheri* Yavuz & Aktaş (2017) found the similar results in terms of Pb concentration (means of ^{206}Pb: 0.38 and ^{208}Pb: 0.28) in muscle tissue from mine active area in Turkey. On the other hand, Pb accumulation levels in muscle tissue of wild rodent species from different polluted areas in world higher than our results The values are; 2.14 ppm for *Rombomys opimus* and 2.51 ppm for *Rattus norvegicus* from different land uses in Iran, 3.60 ppm for *Clethrionomys glareolus* that collected from abandoned lead mine in UK, 7.17 ppm for *Acomys cahirinus* which collected from polluted area in Egypt, 32.63 ppm for *Peromyscus maniculatus* and 44.95 ppm for *Microtus pennsylvanicus* that collected from oil sands and surface mines in Canada. (Milton et al., 2003; Mohallal and Younes 2015; Rodriguez-Estival & Smits, 2016; Zarrintab & Mirzaei, 2017).

Data obtained in the current study indicate that the accumulation values of Co ($\overline{X}$= 0.21±0.08) correlated with some literature data. Literature values are; 0.11 ppm for *Microtus guentheri* obtained from relatively clean area and 0.21 ppm for *Microtus guentheri* from near active mine sites in Turkey at the same study (Yavuz & Aktaş, 2017). But according to another study, Co accumulation values in muscle tissue are quite higher than our results. These values are; 2.28 ppm for *Microtus pennsylvanicus* and 4.07 ppm for *Peromyscus maniculatus* from oil sands and surface mines in Canada (Rodriguez-Estival & Smits, 2016)

Data obtained in the current study indicate that the accumulation values of Cr and Mn are higher than literature values. For values obtained for Cr, the accumulation concentration value ($\overline{X}$=7.19±1.43 ppm) is quite higher than the literature values for rodent muscle tissue. The values are; 0.61 ppm from *Peromyscus maniculatus* and 0.34 ppm from *Microtus pennsylvanicus* from oil sands and surface mines in Canada (Rodriguez-Estival & Smits, 2016) and 0.50 ppm for muscle of

Peromyscus gossypinus which collected from different contaminated areas in USA (Reinhart, 2003) and 0.56 ppm for *Microtus guentheri* from active mine sites in Turkey and 0.55 ppm for clean area in Turkey (Yavuz & Aktaş, 2017). Similarly, obtained for Mn from muscle tissue of *M. guentheri* the accumulation concentration value ($\overline{X}$=5.97±1.42 ppm) is higher than the literature values: the values are 1.23 ppm for *Peromyscus maniculatus* and 1.26 ppm for *Microtus pennsylvanicus* from oil sands and surface mines in Canada (Rodriguez-Estival & Smits, 2016), 2.05 ppm for *Acomys cahirinus* on polluted areas from Egypt (Mohallal & Younes, 2015) and 0.97 ppm for muscle of *Peromyscus gossypinus* in different contaminated areas in USA (Reinhart, 2003) and 3.19 ppm for *Microtus guentheri* from active mine sites in Turkey and 1.21 ppm from clean area in Turkey (Yavuz & Aktaş, 2017).

For values obtained for Cd, the accumulation concentration value ($\overline{X}$=0.001±0.0008 ppm) is lower than the literature values for rodent muscle tissue. The values are; 0.69 ppm for *Microtus guentheri* from near active mine sites and 0.48 ppm for relatively clean area in Turkey (Yavuz & Aktaş, 2017), 0.47 ppm for *Rombomys opimus* different land uses with city Iran (Zarrintab & Mirzaei, 2017), 0.41 ppm for *Sorex araneus* and 0.13 *ppm* for *Clethrionomys glareolus* from forest containing ash in Finland (Lodenius et al,. 2002), 0.07 ppm for *Heliophobius argenteocinereus* city's different habitats in Malawi (Umbera et al., 2003), 0.04 ppm for *Peromyscus gossypinus* pollutes areas in USA (Reinhart, 2003), 0.03 ppm for *Clethrionomys glareolus* near active mine sites in Ireland (Milton & Johnson, 1999).

This is the first record for revealing the accumulation levels of Sr, Ba, Se, V, Sn, Mo for tissue samples of *M. guentheri* in the mentioned area in Turkey, and there was no literature information for these heavy metals. For this reason, it is expected that the current study may be crucial for eliminating lack of literature in this field. It is anticipated that the elements mentioned above can be important for monitoring studies in future.

The variation of accumulation heavy metals values in literature, it is expected that the types of accumulation differ from each other due to different species of indicator rodents, variability of pollution factors, difference of mining and mining activity type (marble, stone, lead or coal mines etc.) and variation of agricultural pollution type (the difference between selected pesticides and fertilizers etc.).

The aim of this study was to analyse the accumulation levels of heavy metals in muscle samples of *Microtus guentheri* inhabiting in polluted sites in Korkuteli-Antalya. The obtained results allow us to assess the role of the *M. guentheri* as a bioindicator of heavy metal pollution. Prior to this study there was no information available about the heavy metal levels of small mammals in this region. In this field, stone quarries and mines are located. For this reason, the primary objective of this study was to measure in the present levels of heavy metal accumulation that could be used to examine future environmental effects of bioaccumulation from the mines and marble quarries. Our results are the first study of this species living in this region. On the other hand, our bioindicator species *M. guentheri* seems to be a suitable bioindicator of metals. The results obtained this study can be used as a reference in the future if bioaccumulation observations are made at different localities. The species examined in this case can be used as a biomonitor for long-term and large-scale studies in the future.

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