HEAVY ELEMENT ACCUMULATION IN SOME *AGABUS* SPECIES (COLEOPTERA: DYTISCIDAE) FROM DIFFERENT PROVINCES OF TURKEY

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ABSTRACT: During 2007 and 2008, *Agabus* spp. (Dytiscidae) were collected and heavy element content of the insects were evaluated. Heavy element concentrations were analyzed by Energy Dispersive X-Ray Fluorescence (EDXRF) spectroscopy. In this study sixteen heavy element (Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Br, Rb, Sr, Pb) were measured for heavy element pollution in different city (Afyon, Denizli, Kütahya, Uşak) of Turkey. According to the results insects are able to accumulate these elements in certain concentrations.

KEY WORDS: Bioaccumulation, Dytiscidae, *Agabus*, EDXRF, heavy element, Turkey

Heavy elements are important environmental pollutant and are worldwide problem in these days. Elements are part of earth crust but unconscious and intensive usage of these elements increase the availability in ecosystem and food chain. Minor amount of those elements are necessary for continue life. But increasing level of heavy element affects drinking water quality, environment, food web and finally human health (Onuoha & Felicia, 2008).

Aquatic beetles are diverse group of Arthropoda, spending their part of life cycle within water and so they are part of different kind of water bodies and wetlands (Pennak, 1978; Hansen, 1987). They not only serve as food for fish, amphibians and water birds but also they decompose organic matters and nutrients (Rosenberg & Resh, 1993). They are often utilized in ecosystem research to assess the ecosystem quality (Brinkman & Johnston, 2008; Aydoğan et al., 2017) because they cover wide areas during their foraging activity.

Dytiscidae also known as diving beetles are well adapted to aquatic life. Their lifecycle, both larval and adult stages, spend in the water, especially near vegetation. Larvae generally sink in the water whereas adults are positively buoyant (Miller & Bergsten, 2016). Dytiscidae are central to aquatic food webs and are one of the most diverse and important groups of aquatic predatory insects. During their life cycle they are feeding carnivorously at least part of their life. Larvae are predaceous whereas adults are carnivorous and also feed as scavengers (Culler et al., 2014; Miller & Bergsten, 2016). Agabinae belongs to Dytiscidae and lives in many habitats including lentic and lotic water bodies. The objective of this study was to determine the heavy element accumulation levels by the Agabinae beetles.

MATERIAL AND METHODS

Field-work was carried out in the spring and summer (April-June) of 2007-2008. All samples were collected from freshwater habitats of four different cities (Afyon, Denizli, Kütahya and Uşak) of Turkey. The data about the sampled sites are given in Table 1 with description of the exact GPS coordinates, altitude, location name and site description.

Collection of samples. The samples were collected by means of a sieve with 1 mm pores from the shallow areas of various springs, streams, lakes, ponds, brook and puddles. The beetles were killed with ethyl acetate and were stored in small bottles until identification and taken back to the laboratory for analysis. They were preserved in 95% alcohol, which was replaced by 75% alcohol and 5% glycerin mix after 24 hours. Specimens were cleaned with brush before identification. They were sorted on a petri dish and identified to the specie level using taxonomic keys. Aedeagophores of collected specimens were dissected under a stereo microscope in the laboratory. Aedeagophores of the beetles cleaned with brushes, were dissected under a stereo microscope and left in a 10% KOH solution for 1-2 h. Seven species belonging to genus *Agabus* Leach, 1817 were identified. These species are as follows; *Agabus biguttatus* (Olivier, 1795), *Agabus nebulosus* (Forster, 1771), *Agabus conspersus* (Marsham, 1802), *Agabus guttatus* (Paykull, 1798), *Agabus didymus* Olivier, 1795, *Agabus bipustulatus* (Linnaeus ,1767), *Agabus labiatus* (Brahm, 1790).

Elemental analysis. In this study, insect samples were analyzed as described in Aydoğan et al. (2016, 2017). Quantity analysis of 16 elements in insect samples was measured by Energy Dispersive X-Ray Fluorescence (EDXRF) spectrometry. When insect identification completed the samples were dried at 80˚C during 36 h using a microwave. Insects were pulverized and then cellulose was added in order to gain a better shape. Five tons of pressure applied to make 13 mm diameter tablets of each species. 13 mm diameter tablets of insect samples were irradiated by 59.5 keV photons, emitted by 1 Ci ^{241}Am radioactive source. X-ray spectra were collected with HPGe detector which use Genie-2000 software (Canberra) program. HPGe detector resolution is ~180 eV. The irradiation time was 43.200 s for insect samples. Source/Sample distance was 35.5 mm. The measurements were carried out under vacuum. The spectral data were stored on disks, and the concentration of elements in each samples were determined by WinAXIL software (Canberra) and Win Fund software package (Canberra), which use the Fundamental Parameters Method (FPM) for quantitative analysis. The model parameters are then optimized by means of a non-linear least squares strategy, using a modified Marquardt algorithm to minimize the weighted (optional choice) sum of differences $\chi 2$ between the experimental data and the mathematical model. Elemental concentrations of the insect samples have some uncertainties due to EDXRF (maximum ~5%). Possible error sources for these uncertainties are listed in Table 2. Typical spectrum of samples in EDXRF was shown in Figure 1.

RESULTS

In the freshwater habitat of four different cities (Afyon, Denizli, Kütahya, Uşak), seven aquatic beetle species belonging to genus *Agabus* (Dytiscidae) were recorded. In insects' total body sixteen heavy elements (Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Br, Rb, Sr, Pb) were measured. All elements were measured in certain amount in all insect samples, *Agabus nebulosus* except to this. Ca, V, Ti, Cr, Mn, Fe did not measured in *Agabus nebulosus*. The results showed that element concentration in all samples have differences, but the value of concentrations nearly same in *Agabus biguttatus*, *Agabus conspersus*, *Agabus guttatus* and *Agabus labiatus*. *Agabus didymus* and *Agabus bipustulatus* are the most heavy element accumulator than the other species. *Agabus didymus* is the best accumulator in terms of Ca, Ti, V, Cu, As, Se, Pb whereas *Agabus bipustulatus* is the best accumulator in terms of Cr, Mn, Fe, Ni, Zn and Br. The concentrations of Sr, Rb and Co are nearly same in this two species. Two of this species were collected in the same habitat and besides to this *Agabus conspersus* was also in the same habitat. But *Agabus conspersus* did not collect the highest value of elements than *Agabus didymus* and *Agabus bipustulatus*.

It is well known that the concentration of elements may differ greatly in a genus and also among its species (Rosenberg & Resh, 1993). These differences in concentration could be due to exposure of the organisms to the element, quality of biotic and abiotic habitats, their cycles of food chain and the tendency of elements to bind to certain molecular groups found within the cells (Laws, 1993). The concentrations of heavy elements in insect samples were given in Table 3.

CONCLUSION

These findings indicate thatit is possible to analyze element concentrations in these aquatic insects. The genus Agabus is able to easily identifiable, have numerical abundance in the monitoring areas and it is cosmopolitan. Therefore, it can be concluded that *Agabus didymus* and *Agabus bipustulatus* accumulate highest level than the other species thus; they are more tolerable for heavy elements and can be used in environmental monitoring or contamination studies. However, further studies are needed to provide data on pollutant accumulation in Dytiscidae. Element accumulation in the examined aquatic beetles currently does not pose a threat to their habitat function. Probably more than any other element, the presence and enrichment of Lead (Pb) is an indicator of anthropogenic pollution. To evaluate environmental quality geologic background, sediment and water heavy element levels also should take into consideration. To assess health of environment, long-term biomonitoring must be regularly done in biotic and abiotic environments.

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Table 1. Collected samples and description of the study areas.

Sample Name	Altitude (m)	Coordinates	Station information	Notes on near environment
Agabus biguttatus	921 m	37°41'294"N 29°37 '290"E	DENİZLİ: Çardak (Beylerli Town-Değirmendere) 22.04.2008	Stream
Agabus nebulosus	834 m	39°06'124"N 29°02'179"E	KÜTAHYA: Simav (Gökçeler Village-Sulama Pond) 21.05.2008	Sand sail, No vegetation
Agabus conspersus	958 m	38°39'331"N 29°46'825"E	UŞAK: Banaz (Ahat Village-Çeşme) 23.06.2007	Puddle
Agabus guttatus	985 m	37°21'878"N 29°20'292"E	DENİZLİ: Acıpayam 27.04.2007	Puddle
Agabus didymus	958 m	38°39'331"N 29°46'825"E	UŞAK: Banaz (Ahat Village-Çeşme) 23.06.2007	Puddle
Agabus bipustulatus	958 m	38°39'331"N 29°46'825"E	UŞAK: Banaz (Ahat Village-Çeşme) 23.06.2007	Puddle
Agabus labiatus	1020 m	38°04'587"N 30°16'505"E	AFYON: Dinar (Karakuyu Lake) 22.05.2008	Pond
	1373 m	39°23'713"N 30°19'218"E	KÜTAHYA: Türkmen Mountain (Söğüt Yaylası-Dere) 18.06.2008	Brook

Table 2. The error sources in the experimental results.

Nature of Uncertainty	Uncertainty (%)
Counting Statistics	~ 1.00
Systematic errors	~2.00
Peak evaluation procedure	~3.00
Fundamental parameter methods	~3.00

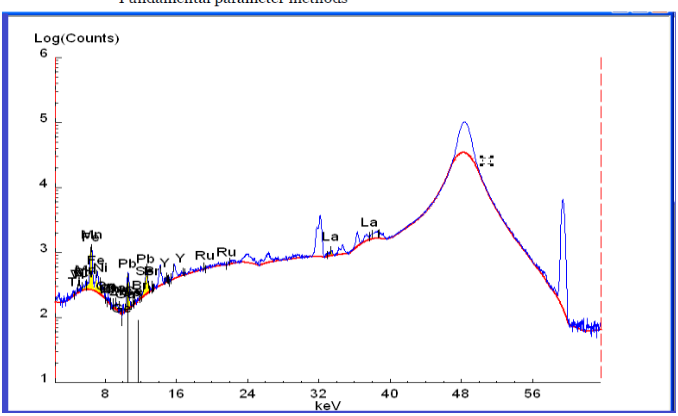

Figure 1. The typical spectrum of samples in EDXRF.

Table 3. Concentration of elements in the genus *Agabus* (Dytiscidae) species (ppm).

Heavy Element	*Agabus biguttatus*	*Agabus nebulosus*	*Agabus conspersus*	*Agabus guttatus*	*Agabus didymus*	*Agabus bipustulatus*	*Agabus labiatus*
Ca	0.714	ND	1.061	1.129	285.2	240.5	06.56
Ti	0.431	ND	0.390	0.446	100.9	75.61	0.399
V	0.267	ND	0.290	0.283	54.64	49.47	0.290
Cr	0.199	ND	0.223	0.252	29.81	44.16	0.227
Mn	0.155	ND	0.234	0.178	21.59	23.46	0.158
Fe	0.117	ND	0.134	0.125	14.49	15.20	0.115
Co	0.156	0.331	0.189	0.170	86.28	86.48	0.152
Ni	0.133	0.286	0.137	0.135	59.64	81.14	0.168
Cu	0.132	0.155	0.110	0.112	61.85	36.74	0.097
Zn	0.087	0.126	0.094	0.093	26.10	30.64	0.078
As	0.223	0.247	0.175	0.183	39.83	29.03	0.148
Se	0.053	0.073	0.049	0.062	8.248	7.650	0.046
Br	0.051	0.071	0.052	0.043	6.489	8.254	0.042
Rb	0.037	0.064	0.040	0.038	4.021	4.048	0.029
Sr	0.031	0.047	0.030	0.030	3.322	3.027	0.028
Pb	0.463	0.510	0.362	0.378	82.74	60.15	0.305

ND: Not detected