THE FIRST ANALYSIS OF HEAVY METALS IN THE GREY HERON Ardea cinerea FEATHERS FROM THE CROATIAN COLONIES

Prva analiza teških metala u perima sivih čaplji Ardea cinerea iz hrvatskih kolonija

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ABSTRACT

This study represents the first analysis of heavy metals: lead and cadmium, as well as two metalloids: arsenic and selenium, in the feathers of Grey Herons breeding in Croatia. The Grey Heron was chosen as a model apex predator of the wetland ecosystem due to high trophic level, feeding habits, long lifespan and abundance. Sampling was conducted during the 2019 breeding season in April and May from six Grey Herons colonies in Croatia (Mrsunjski lug, Kopački rit, Piljenice, Kravarsko, Čepin, Slovinci), whose environment is heavily influenced by rivers Danube, Drava and Sava tributaries. The primary and secondary flight feathers were collected under the colonies as shed feathers or from dead birds. The feathers are a suitable non-invasive method for metal and metalloid analysis, and can reflect internal concentrations, depending on the metal. In the Slovinci colony, we measured the highest lead
(Pb) and cadmium (Cd) concentrations (2194.53 ± 416.20 µg kg⁻¹ dw and 79.30 ± 9.37 µg kg⁻¹ dw, respectively). The highest arsenic (As) and selenium (Se) concentrations were found in the Čepin colony (359.70 ± 301.46 µg kg⁻¹ dw and 3375.61 ± 2502.80 µg kg⁻¹ dw, respectively). All the measured levels of heavy metals and metalloids are below concentrations that can cause adverse health effects in birds.

**Keywords:** biomonitoring, non-invasive sampling, apex predator birds, metal pollution, Croatia

**INTRODUCTION**

Increased input of hazardous substances, such as heavy metals, from anthropogenic sources results in environmental pollution that may cause adverse effects on the surrounding fauna and flora (Janssens et al. 2001). Metal reactivity depends on their different forms in the environment, and the metal-induced toxicity is related to the oxidative status. Unlike organic compounds, metals cannot be metabolized into less toxic compounds, which results in their high persistence (Koivula & Eeva 2010). Analysing heavy metal pollution by using living organisms can provide more reliable results compared to only measuring the metal concentration in the environment (Markowski et al. 2013). Apex predators have proved to be relevant bioindicators for various xenobiotics, such as heavy metals and persistent organic pollutants (POPs) (Eulaers et al. 2011). Due to rapid accumulation of chemicals in the freshwater ecosystem and accumulation of chemicals in the sediment, waterbirds are particularly at risk (Burger & Eichorst 2005), but they can serve as excellent bioindicators of environmental change (Kushlan 1993). In aquatic environment, existing contaminants can biomagnify through the food chain, reaching deleterious concentrations at the highest trophic levels, and causing adverse health effects on reproduction, egg hatchability, hatching survival, and neurobehavioral development (Tsipoura et al. 2011). Considering all this, in the present study, the Grey Heron *Ardea cinerea* was chosen as a model apex predator of the wetland ecosystem. The Grey Heron is the most abundant species of the heron family (Ardeidae) in Croatia, with increasing population since the 1970s (Mikuška et al. 2005, Komljenović 2019). It is a long-lived species with estimated 10.3 years generation length (BirdLife International 2020); they can live up to 37 years in the wild (Fransson et al. 2010). As a typical wetland bird, it is found in freshwater ecosystems, e.g. rivers, lakes, ponds, wetlands, marshes, water reservoirs (Witherby et al. 1949), where it feeds on fish, amphibians, crustaceans, snakes, small mammals and large insects (Hancock & Kushlan 1984). Daily food intake is estimated to be 500 grams of prey per day (del Hoyo et al. 1992). Quite often, particularly during drought or winter, when freshwaters are frozen, it would stalk and feed on small rodents on agricultural land, meadows and pastures. Depending on prey abundance, it can forage solitaire or in groups...
next to other freshwater bird species, such as other heron species, gulls, spoonbills, and storks (Jakubas & Mioduszewska 2005). The breeding colonies, heronries, are usually built close to feeding sites (up to 8 km), but birds can forage up to 20 km from the breeding site (Whiterby 1943). It is a breeding, migratory and wintering bird species in Croatia. It is however hypothesized that a part of the Croatian population is partially resident (Mikuška et al. 2005, Lukač 2007, Kralj et al. 2013). Grey Herons in Croatia start to breed earlier than other heron species, in late March and early April. At the time of breeding, sexually immature birds are not present in the colonies. Grey Herons would undergo partial moulting that would not impact their flight capabilities. Partial post-juvenile moult is confined to most of the body feathers and rarely some wing coverts, while the flight feathers remain unmoulted until their second year (Blasco-Zumeta & Heinze 2020).

The adults finish their post-breeding moult usually in November. Herons are very often used as bioindicator for local contaminations due to their abundance, position in the food chain, and exposure to various hazardous chemicals through their diet containing pollutants that have a tendency to bioaccumulate (Burger 1994, Burger 1995, Scheifler et al. 2006, Burger et al. 2007, Deng et al. 2007, Horai et al. 2007). Feather analysis is an informative indicator for investigating various physiological processes affected by metals. The feathers are suitable monitoring tools for metals and metalloids, especially in top predators, which have a long life span, so that various xenobiotics have time to accumulate (Rutkowska et al. 2018). There are at least three different mechanisms for increasing metal concentration in the feather: internal deposition during growth, which reflects internal contamination, contamination by secretions from preen oil gland, and external contamination (Goede & De Bruin 1986). The feathers can be collected in a non-invasive way, stored at room temperature, and easily transported (Eulaers et al. 2011). Metal variation in the feather can be attributed to several factors, such as moulting pattern, feather segment, feather formation, rate of feather growth, leaching, pigmentation and external contamination (direct or from preen gland) (Goede & De Bruin 1984). The feathers are good indicators of exposure to arsenic and lead. Selenium levels in the feathers reflect the internal exposure history of the bird (Goede 1985, Burger 1993, Ansara-Ross et al. 2013). The feathers are furthermore reliable biomonitoring tools for cadmium exposure, as they are correlated with internal concentration in liver, kidney and uropygial gland (Pilastro et al. 1993). Although the Grey Heron feathers have already been used around the world for heavy metal biomonitoring, no such study on the feathers has been completed in Croatia. The main objective of this study is to use the feathers as a non-destructive biomonitoring matrix, and assess the concentrations of heavy metals – lead and cadmium, as well as two metalloids – arsenic and selenium. This study represents the first analysis of metals and metalloids in the feathers of the Grey Heron breeding in Croatia.
MATERIALS AND METHODS

The sampling was conducted during the 2019 breeding season in April and May. The feathers were collected under the colonies from six different locations: Mrsunjski lug \((n=4)\), Kopački rit \((n=4)\), Piljenice \((n=3)\), Kravarsko \((n=3)\), Čepin \((n=2)\), and Slovinci \((n=2)\) (Figure 1). Eastern sampling locations include three colonies: Kopački rit, Čepin and Mrsunjski Lug. Čepin is a small colony with 46 pairs, Mrsunjski Lug is a medium colony with 148 pairs, while Kopački rit is a large colony with 424 pairs (Komljenović 2019). Their environment and climate are influenced by three major rivers – Danube, Drava and Sava. Danube and Drava construct major floodplains and wetlands, also known as the Kopački rit Nature Park (Sić 1975, Blagojević 2008). Central sampling locations include Kravarsko, Piljenice and Slovinci. Kravarsko and Slovinci are medium colonies with 55 and 64 pairs, respectively. Piljenice is a large colony with 325 breeding pairs (Komljenović 2019). Central Croatia is heavily influenced by Sava tributaries: Sutla, Una, Kupa, Sunja, Lonja, Češma and Ilova, as well as by Drava tributaries: Plitvica, Bednja, Gliboki potok, Mura and Trnava (Crkvenčić 1974). The primary and secondary flight feathers were collected from adult birds either as shed feathers or from dead individuals found under the colony. The feathers had been stored in paper envelopes before the analysis. The feathers were analysed for the following elements: lead (Pb), arsenic (As), cadmium (Cd), and selenium (Se). Prior to the analysis, all the feather samples had been washed with deionized water and ground to fine powder using a heavy-metal-free mill, following digestion with a 10 mL 5:1 mixture of \(\text{HNO}_3\) (Trace metal grade, Fischer) and \(\text{H}_2\text{O}_2\) (Primar–trace metal grade, Fischer) in a microwave oven (CEM Mars 6, USA). The concentrations of metals and metalloids were measured using ICP–MS (Agilent 7500a Inductively Coupled Plasma Mass Spectrometer). All the feather samples were measured in duplicate with Chicken – Trace elements NCS ZC 73016 as reference material. The limit of detection for the analysed elements is as follows: 0.02 \(\mu g\) kg\(^{-1}\) for lead, 0.04 \(\mu g\) kg\(^{-1}\) for arsenic, 0.0004067 \(\mu g\) kg\(^{-1}\) for cadmium, and 0.06563 \(\mu g\) kg\(^{-1}\) for selenium. All the analyses were conducted at the Faculty of Agrobiotechnical Sciences in Osijek, Croatia. Table 1 shows the concentrations for each sample, as well as the arithmetic mean (\(\mu g\) kg\(^{-1}\) dry weight) \(\pm SD\) for each location. Figures 2-5 show the data by boxplots (the line in the middle of the box representing the median, the box representing the minimum and the maximum value).
RESULTS AND DISCUSSION

Feather analysis has many advantages. i.e. it is easy to collect a sufficient amount of samples and to store the samples; the metal and metalloid concentrations reflect internal concentration during feather growth due to dietary intake, or they can pinpoint local contamination (Jaspers et al. 2019). Furthermore, metal and metalloid concentrations in the feathers represent long-term contamination processes. When interpreting the results, it is important to take into account external contamination, e.g. atmospheric dust, water or contaminant deposition during preening, and geochemical data (Borghesi et al. 2017). Lead, arsenic, cadmium and selenium were detected in all the measured feather samples.

The highest lead concentration was observed in Slovinci (2194.53 ± 416.20 µg kg⁻¹ dw). Surprisingly, a relatively high concentration of lead was observed in Kopački rit as well (1955.85 ± 1373.07 µg kg⁻¹ dw). All the measured concentrations were below the toxicity level. Adverse effects in birds occur at levels of 4000 µg kg⁻¹ of lead in feathers (Custer & Hoffman 1994, Tsioura et al. 2011, Burger 2013). Lowest lead concentration was measured in Piljenice (587.77 ± 330.18 µg kg⁻¹ dw) (Table 1; Figure 2).
Table 1. Heavy metal and metalloid concentrations (mean ± SD, µg kg⁻¹ dw) in the Grey Heron feathers from six colonies in Croatia.

<table>
<thead>
<tr>
<th></th>
<th>Pb</th>
<th>As</th>
<th>Cd</th>
<th>Se</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mrsunjski lug</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n = 4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1376.25</td>
<td>166.99</td>
<td>23.81</td>
<td>1400.87</td>
<td></td>
</tr>
<tr>
<td>645.30</td>
<td>222.19</td>
<td>8.75</td>
<td>1569.14</td>
<td></td>
</tr>
<tr>
<td>753.45</td>
<td>174.04</td>
<td>13.90</td>
<td>3481.69</td>
<td></td>
</tr>
<tr>
<td>913.80</td>
<td>136.47</td>
<td>21.91</td>
<td>2173.58</td>
<td></td>
</tr>
<tr>
<td>mean ± SD</td>
<td>922.20 ± 298.62</td>
<td>174.92 ± 33.19</td>
<td>17.09 ± 7.56</td>
<td>2156.32 ± 873.87</td>
</tr>
<tr>
<td>Kopački rit</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n = 4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1111.90</td>
<td>165.67</td>
<td>43.87</td>
<td>2763.72</td>
<td></td>
</tr>
<tr>
<td>1047.80</td>
<td>361.11</td>
<td>23.75</td>
<td>1339.62</td>
<td></td>
</tr>
<tr>
<td>1504.70</td>
<td>197.90</td>
<td>14.65</td>
<td>1298.68</td>
<td></td>
</tr>
<tr>
<td>4159.00</td>
<td>278.88</td>
<td>106.01</td>
<td>1181.47</td>
<td></td>
</tr>
<tr>
<td>mean ± SD</td>
<td>1955.85 ± 1373.07</td>
<td>250.89 ± 81.40</td>
<td>47.07 ± 38.74</td>
<td>1645.87 ± 692.86</td>
</tr>
<tr>
<td>Piljenice</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n = 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1008.35</td>
<td>195.14</td>
<td>21.23</td>
<td>1299.33</td>
<td></td>
</tr>
<tr>
<td>328.65</td>
<td>380.94</td>
<td>8.38</td>
<td>1890.35</td>
<td></td>
</tr>
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<td>426.30</td>
<td>121.19</td>
<td>11.02</td>
<td>2637.81</td>
<td></td>
</tr>
<tr>
<td>mean ± SD</td>
<td>587.77 ± 330.18</td>
<td>232.42 ± 119.75</td>
<td>13.54 ± 6.60</td>
<td>1942.49 ± 600.38</td>
</tr>
<tr>
<td>Location</td>
<td>Sample Size</td>
<td>Pb</td>
<td>As</td>
<td>Cd</td>
</tr>
<tr>
<td>------------</td>
<td>-------------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
</tr>
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<td>Kravarsko</td>
<td>n = 3</td>
<td>433.70</td>
<td>147.79</td>
<td>19.11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2153.30</td>
<td>66.63</td>
<td>88.24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1242.10</td>
<td>72.81</td>
<td>73.81</td>
</tr>
<tr>
<td><strong>mean ± SD</strong></td>
<td></td>
<td>1276.37 ± 769.55</td>
<td>95.74 ± 40.48</td>
<td>60.39 ± 32.65</td>
</tr>
<tr>
<td>Čepin</td>
<td>n = 2</td>
<td>672.40</td>
<td>620.76</td>
<td>77.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>618.20</td>
<td>98.64</td>
<td>18.76</td>
</tr>
<tr>
<td><strong>mean ± SD</strong></td>
<td></td>
<td>645.30 ± 31.34</td>
<td>359.70 ± 301.46</td>
<td>47.90 ± 33.66</td>
</tr>
<tr>
<td>Slovinci</td>
<td>n = 2</td>
<td>2554.75</td>
<td>107.07</td>
<td>87.41</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1834.30</td>
<td>108.97</td>
<td>71.19</td>
</tr>
<tr>
<td><strong>mean ± SD</strong></td>
<td></td>
<td>2194.53 ± 416.20</td>
<td>108.02 ± 1.74</td>
<td>79.30 ± 9.37</td>
</tr>
</tbody>
</table>
Lead is widely distributed in the environment with the most relevant source being lead ammunition, causing lead poisoning especially in waterfowl, apex predators and scavengers (García-Fernández 2014). Lead accumulates primarily in bones and kidneys, and is greater in females, than in males and young birds (Koivula & Eeva 2010). Lead in soil is mostly found by the course of the river Drava as a result of anthropogenic activities, as well as in alluvial plains, and the confluence of the rivers Drava and Danube (Halamić et al. 2009, Šajn et al. 2011, Halamić et al. 2012). From soil, lead can adsorb to sediment, and leach into rivers, affecting every trophic level (Millot et al. 2004). According to Goede & De Bruin (1986), lead can be deposited in the preen gland secretion of the birds. After feather formation, the feather can give indirect information about exposure to lead. According to Grúz et al. (2019), the highest concentration in the feathers of predatory birds in Hungary was 2300 µg kg\(^{-1}\) dw, which does not indicate poisoning. Lead does not generally biomagnify through the food chain, therefore high lead levels may indicate proximity to contaminated sites or secondary poisoning through lead-poisoned prey or carcasses (Lee 2003, Legagneux et al. 2014,
Ali & Khan 2019, Grúz et al. 2019). Lead ammunition is widely used in hunting in Croatia and is causing acute and chronic lead poisoning, confirmed in scavenger species, as the Griffon Vulture (Gyps fulvus) (Tišlar et al. 2019).

The measured levels of arsenic ranged from 95.74 ± 40.48 µg kg⁻¹ dw in Kraparsko to 359.70 ± 301.46 µg kg⁻¹ dw in Čepin (Table 1; Figure 3). All the measured levels were below the level of adverse biological effect of 2000–10,000 µg kg⁻¹ dw (Eisler 1988).

**Figure 3.** Boxplot of arsenic (As) concentrations (µg kg⁻¹ dw) in the Grey Heron feathers from six colonies in Croatia (median, min–max).

**Slika 3.** Koncentracija arsena (As) (µg kg⁻¹ suhe tvari) u perima sivih čaplji iz šest kolonija u Hrvatskoj (medijan, min–max).

Arsenic has the ability to bioaccumulate, especially in its inorganic form, and it can affect reproduction (Koivula & Eeva 2010). Arsenic concentration in the feathers reflects environmental pollution well; however, it does not correspond with internal levels (Geens et al. 2010). Arsenic concentration in severely polluted areas can be as high as 30,000 µg kg⁻¹ in passerine birds (Janssens et al. 2001). According to Sánchez-Virosta et al. (2015), arsenic concentrations are below 1000 µg kg⁻¹ in unpolluted areas, and below 10,000 µg kg⁻¹ in polluted areas. The highest recorded arsenic level is 359.70 µg kg⁻¹ (in Čepin), lower than the one mea-
sured in Hungary of the bird of prey feathers (400 µg kg\(^{-1}\) dw) according to Grúz et al. (2019), suggesting relatively unpolluted sampling areas in Croatia. According to Luo et al. (2015) and Eisler (1988), arsenic is accumulated in the organisms, yet it does not biomagnify through the food chain. When compared to ecologically similar species in Table 2, the Cape Cormorant (Phalacrocorax capensis), the Lesser Flamingo (Phoeniconaias minor) and the Crested Ibis (Nipponia nippon) had considerably higher arsenic concentrations in their feathers. This may be due to different feeding habits, and the sampling locations were close to agricultural activities (e.g. pesticide, herbicide and fertilizer use are known as arsenic sources).

The highest recorded concentration of cadmium was 79.30 ± 9.37 µg kg\(^{-1}\) dw in the Slovinci colony (Table 2, Figure 4). In all the colonies, cadmium levels were considerably lower than the adverse biological effect level of 2000 µg kg\(^{-1}\) (Eisler 1985), with the lowest measured concentration in Piljenice (13.54 ± 6.60 µg kg\(^{-1}\) dw).

Figure 4. Boxplot of cadmium (Cd) concentrations (µg kg\(^{-1}\) dw) in the Grey Heron feathers from six colonies in Croatia (median, min–max).

*Slika 4. Koncentracija kadmija (Cd) (µg kg\(^{-1}\) suhe tvari) u perima sivih čaplji iz šest kolonija u Hrvatskoj (medijan, min–max).*
Table 2. Comparison of concentration of heavy metal and metalloids, number of samples (n), locations in the Grey Heron and ecologically similar species. All values are reported as mean values (µg kg\(^{-1}\) dw) from adult flight feathers (shed or from dead birds).

<table>
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<tr>
<th>Scientific name</th>
<th>Species</th>
<th>n</th>
<th>Location</th>
<th>Pb</th>
<th>As</th>
<th>Cd</th>
<th>Se</th>
<th>Reference</th>
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<tr>
<td>Ardea cinerea</td>
<td>Grey Heron</td>
<td>18</td>
<td>Croatia</td>
<td>1266</td>
<td>201</td>
<td>41</td>
<td>2024</td>
<td>This study</td>
</tr>
<tr>
<td>Phalacrocorax capensis</td>
<td>Cape Cormorant</td>
<td>6</td>
<td>Namibia</td>
<td>4340</td>
<td>910</td>
<td>1420</td>
<td>1470</td>
<td>Burger &amp; Gochfeld 2001</td>
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<tr>
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<td>Lesser Flamingo</td>
<td>8</td>
<td>Namibia</td>
<td>386</td>
<td>976</td>
<td>38</td>
<td>841</td>
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</tr>
<tr>
<td>Mycteria americana</td>
<td>Wood Stork</td>
<td>16</td>
<td>Costa Rica</td>
<td>2230</td>
<td>NA*</td>
<td>206</td>
<td>3430</td>
<td>Burger et al. 1993</td>
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<tr>
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<td>Little Egret</td>
<td>7</td>
<td>India</td>
<td>2645</td>
<td>NA</td>
<td>245</td>
<td>NA</td>
<td></td>
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<tr>
<td>Bubulcus ibis</td>
<td>Cattle Egret</td>
<td>3</td>
<td>India</td>
<td>3845</td>
<td>NA</td>
<td>1900</td>
<td>NA</td>
<td>Muralidharan et al. 2004</td>
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<tr>
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<td>6205</td>
<td>NA</td>
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<tr>
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<td>4</td>
<td>India</td>
<td>NA</td>
<td>NA</td>
<td>250</td>
<td>NA</td>
<td>Mirsanjari et al. 2014</td>
</tr>
<tr>
<td>Phalacrocorax carbo</td>
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<td>Iran</td>
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<td>NA</td>
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<tr>
<td>Nipponia nippon</td>
<td>Crested Ibis</td>
<td>13</td>
<td>China</td>
<td>390</td>
<td>1000</td>
<td>110</td>
<td>180</td>
<td>Liu et al. 2019</td>
</tr>
<tr>
<td>Egretta garzetta</td>
<td>Little Egret</td>
<td>10</td>
<td>Hong Kong</td>
<td>2067</td>
<td>NA</td>
<td>57</td>
<td>NA</td>
<td>Connell et al. 2002</td>
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<tr>
<td>Nycticorax nycticorax</td>
<td>Black-crowned Night Heron</td>
<td>2</td>
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<td>1650</td>
<td>NA</td>
<td>50</td>
<td>NA</td>
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*NA – not analyzed
Birds are exposed to cadmium primarily throughout their diets, and the levels are influenced by their feeding habits, age, physiological status, etc. Chronic exposure to cadmium reduces reproductive success (García-Fernández 2014). Uneven cadmium distribution on the Croatian soil is reported by Halamić et al. (2009), as well as in the Podravina region. Higher cadmium concentrations in the air were reported in the flood plain sediments of the rivers Drava and Mura according to Vučković et al. (2012). Higher cadmium content in Croatia is attributed to the mining activities in Slovenia (Mezica Pb-Zn mine) and Austria (Pb-Zn mine with Cd) (Šajn et al. 2011). However, cadmium concentrations measured in the Grey Heron feathers were rather low on all locations when compared to the levels measured in the Eurasian Oystercatcher (Haematopus ostralegus) of 180 µg kg⁻¹ from the Wadden Sea (Stock et al. 1989), and the birds of prey in Hungary, with the recorded concentration of 200 µg kg⁻¹ dw (Grúz et al. 2019). In polluted areas, cadmium levels may rise up to 200 µg kg⁻¹ dw, measured in the feathers of the Great Tit (Parus major) (Dauwe et al. 2004), suggesting a relatively unpolluted environment in Croatia.

Selenium in the flight feathers of Grey Herons ranged from 127.18 ± 222.92 µg kg⁻¹ dw in Slovinci to 3375.61 ± 2502.80 µg kg⁻¹ dw in Čepin (Table 1, Figure 5). All the measured concentrations were below the levels that may cause adverse health effects (3800 µg kg⁻¹ dw) (Burger 2013).

Figure 5. Boxplot of selenium (Se) concentrations (µg kg⁻¹ dw) in the Grey Heron feathers from six colonies in Croatia (median, min–max).

Slika 5. Koncentracija selena (Se) (µg kg⁻¹ suhe tvari) u perima sivih čaplji iz šest kolonija u Hrvatskoj (medijan, min–max).
Selenium plays a biological role in birds; however, it is very embryotoxic at high levels, causing developmental abnormalities with visible malformations in embryos and chicks (García-Fernández 2014). Goede & De Bruin (1986) report that selenium contamination source is external, that it comes mostly from the preen gland secretions, and increases considerably in the course of time. Selenium may cause toxicity in the range 3800–26,000 µg kg\(^{-1}\) (Burger 2013). According to Heinz (1996), selenium levels of 1800 µg kg\(^{-1}\) may cause sub-lethal adverse effects. Selenium is known to accumulate in the aquatic food chain (Maier et al. 1998). Nevertheless, our results indicate relatively unpolluted continental regions in Croatia.

The basic assumption is that Grey Herons get exposed to heavy metals and metalloids through their diet and food source. While the fish fauna of Sava, Drava and Danube is relatively well known (Mrakovčić et al. 2006), and heavy metals and metalloids were identified as pollutants in the Danube river and its tributaries (László 2014, ICPDR 2015), there is only one study on biomonitoring heavy metals in fish in the Croatian part of Danube (Zrnčić et al. 2012). The contamination of lead and cadmium in fish from Danube was analysed, and there was significant difference of the mean concentrations of the measured values according to feeding habits (Zrnčić et al. 2012). According to Zrnčić et al. (2012), lead ranged from 0.015 µg\(^{-1}\) dw in planktivorous to 0.039 µg\(^{-1}\) dw in herbivorous fish, and cadmium from 0.013 µg\(^{-1}\) dw in herbivorous to 0.018 µg\(^{-1}\) dw in piscivorous fish. All of these concentrations were below the national and EU-permitted levels.

As shown in Table 2, only a minor number of studies assessed metal concentration in the flight feathers in adult similar bird species. When compared with the results of other studies, our preliminary data indicate relatively low metal and metalloid content in Grey Herons. However, the source of the metals deposited in the feather cannot be determined. According to Fu et al. (2013), the flight feathers of cranes and herons from a polluted province in China had higher levels of lead, arsenic and cadmium than herons from Croatia. Ansara-Ross et al. (2013) also reported higher levels of arsenic and lead, cadmium levels are similar, and selenium levels are lower in the primary feathers of the African Grass-owl (Tyto capensis).

Preliminary results of this study indicate that the average levels of lead, arsenic and cadmium are well below the toxic levels for all sampled locations. Taking this into account, we conclude that reported values show relatively low metal and metalloid content in Continental Croatia. However, considering scarce data on the Grey Heron post-breeding dispersion and unknown heavy metal and metalloid sources, we can only assume low environmental pollution. Our study contains preliminary results suggesting further investigation is needed on a larger sample size, as well as an establishment of continuous pollutant biomonitoring of predatory bird species in Croatia.
References


SAŽETAK

Antropogenim aktivnostima povećava se razina metala i polumetala u okolišu rezultirajući njihovim zagađenjem. Mjerenje teških metala i polumetala neinvazivnim metodama dobar je pokazatelj stanja okoliša. U radu su predstavljeni rezultati prve analize teških metala: olova i kadmija te dva metaloida: arsena i selena u perju sivih čaplja Ardea cinerea koje se gniježde u Hrvatskoj. Uzorkovanje pera sivih čaplji provedeno je tijekom sezone gniježdenja, u travnju i svibnju 2019. iz šest hrvatskih kolonija (Mrsunjski lug, Kopački rit, Piljenice, Kravarsko, Čepin i Slovinci). Primarna i sekundarna letna pera odraslih ptica prikupljena su ispod kolonija. Najveće koncentracije olova (Pb) zabilježene su u perju iz Slovinaca (2194,53 ± 416,20 µg kg⁻¹ suhe tvari) i Kopačkog rita (1955,85 ± 1373,07 µg kg⁻¹ suhe tvari). U Čepinu su izmjerene najviši koncentracije arsena (As; 359,70 ± 301,46 µg kg⁻¹ suhe tvari) i selena (Se; 3375,61 ± 2502,80 µg kg⁻¹ suhe tvari), a najviša razina kadmija (Cd) izmjerena je u koloniji Slovinci (79,30 ± 9,37 µg kg⁻¹ suhe tvari). Sve izmjerene razine meta-
lai i polumetala su ispod koncentracija koje se smatraju toksičnima za ptice. Preliminarni rezultati ovog istraživanja ukazuju na relativno nisku zagađenost okoliša kontinentalne Hrvatske, no potrebna su dodatna istraživanja i uspostava kontinuiranog praćenja metala i polumetala u okolišu te njihov utjecaj na vršne predatore.