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# ESSENTIAL TRACE (Zn, Cu, Mn) AND TOXIC (Cd, Pb, Cr) ELEMENTS IN THE LIVER OF BIRDS FROM EASTERN POLAND

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*We have focused our study on the concentrations of essential heavy metals (Zn, Cu and Mn) and non-essential trace metals (Pb, Cd and Cr) in the livers of birds from Eastern Poland. The largest mean amount of Zn – as much as 279 mg/kg dry mass (d.m.) – was found in mute swans. However, only in one of the analysed buzzard specimens the concentration of Zn, found to be 664 mg/kg d.m., exceeded the level indicative of poisoning for this element. Birds specializing in catching rodents accumulated Mn in their livers in a very narrow range of concentrations, around 5.0 mg/kg d.m. on average. The range of mean Mn concentrations (around 6.5 mg/kg d.m.) was also found to be narrow for piscivorous birds. The highest mean levels of Pb were found in mute swans (2.7 mg/kg d.m.), and the highest levels of Cd (2.0 mg/kg d.m.) for rooks. Concentrations of total Cr above detection level were found in 22 specimens (53.7%), and concentration values were highest for rooks. Analyses showed that the concentrations of biogenic elements did not exceed the levels indicative of poisoning (except in one specimen). The study demonstrated that lead shots remain a hazard to water ecosystems. Pb, Cd and Cr levels in the livers of omnivorous and piscivorous species indicate the permanent presence of these elements in the environment and may confirm the thesis about the growing role of electronic waste, including metallic e-waste, in the emission of the total amount of contamination with these elements.*

*Key words: atomic absorption spectrometry, birds, Eastern Poland, liver, toxic elements, trace element*

## INTRODUCTION

Free-living birds remain a good indicator of environmental hazards since they often constitute the final link in food chains, as a result of which their organisms can accumulate trace elements, regarded as essential to life, as well as toxic elements. The concentrations of these essential trace and toxic elements in the organs of each species of birds depend on a number of factors. The most typical appear to be: the position in the food chain, diet, the place and manner of foraging, general health condition, age and susceptibility to the absorption of a given element (Kabata-Pendias and Pendias, 1993; Battagalia *et al.,* 2005; Pain *et al*., 2004; Rothschild and Duffy, 2005; Kalisin´ska *et al*., 2004; 2007). Among essential trace elements, those that deserve attention are zinc (Zn), copper (Cu) and manganese (Mn), whose physiological functions in the organisms of vertebrates are fundamental (Kabata-Pendias and Pendias, 1993). Among toxic elements, lead (Pb), cadmium (Cd) and chromium (Cr) deserve particular attention. The former two elements have such a long biological half-life that they accumulate in the body with age and with increasing levels of exposure in the environment (Garcia-Fernandez *et al*., 1995; Clark and Scheuhammer, 2003). The latter one, chromium, may be an important trace element or a toxic one for living organisms, depending on the oxidation state (Kimbrougha *et al*., 1999; Bielicka *et al*., 2005).

The aim of the paper was to present the results of a study undertaken to determine the concentrations of three toxic heavy metals: Pb, Cd and Cr, as well as three biogenic metals: Zn, Mn and Cu, in the livers of free-living species of birds representing the food guilds of Eastern Poland.

## MATERIAL AND METHODS

The studied specimens were livers of 41 specimens of 12 species of birds, representing various food guilds: Bittern *Botaurus stellaris,* Grey heron *Ardea cinerea,* Black stork *Ciconia nigra,* Mute swan *Cygnus olor,* Goosander *Mergus merganser,* Marsh harrier *Circus aeruginosus,* Goshawk *Accipiter gentilis,* Sparrowhawk *Accipiter nisus,* Common buzzard *Buteo buteo,* Common kestrel *Falco tinnunculus*, Long-eared owl *Asio otus* and Rook *Corvus frugilegus*. The number of specimens examined and the classification of each species into food guilds are given in Tables 1 and 2. Livers used in this study were taken mainly from wounded birds brought to rehabilitation centres or veterinary clinics between 2009 and 2010. The birds either died despite having received intensive treatment or, due to being untreatable upon delivery, were put down with a lethal injection by veterinary doctors to spare them suffering. The birds' overall stay in rehabilitation centres/clinics did not exceed 7 days. Some of the birds were found recently killed next to busy roads, windmills and very high buildings, as well as near high-voltage lines or large dumpsites. Others were found near their nesting sites – this refers especially to colony birds (grey herons, rooks). Still other birds had, in some cases, lost their lives in somewhat surprising circumstances. For example, an examined specimen of bittern was found killed in collision with the window of one of the supermarkets in the town of Biala Podlaska. A majority of the examined birds originated from rural, aquatic and forest habitats of Eastern Poland (Bialystok, Lublin and Rzeszów regions). Livers, after their extraction from the birds’ bodies, were stored in freezers until analysis was performed, and finally freeze-dried (lyophilized). Element contents in samples were determined using atomic absorption spectroscopy technique with flame excitation. For this purpose, samples of about 1 g of freeze-dried material were taken and boiled with concentrated nitric(V) acid. After decomposition, the sample solution was filtered out in order to separate it from insoluble remains and diluted to match the measurement sensitivity range. Measurements were performed with the Spectr AA 880 (Varian) spectrophotometer; each measurement was calibrated against standard solutions prepared for each of the relevant elements. Measurement results are given in mg/kg d.m. (dry mass) ± SD (standard deviation).

## RESULTS

*Essential elements*

The hepatic concentration of zinc in one (2.4%) out of 41 examined birds (a specimen of common buzzard) exceeded the level of 525 mg/kg d.m. (i.e. about 150 mg/kg wet mass), which is considered to be the level of Zn indicative of poisoning. Other examined specimens of this species accumulated less zinc and therefore the mean concentration level for the examined buzzards was 121.5 mg/kg d.m. (Table 1). As regards other birds, the mean levels of zinc accumulated in their livers ranged from 53.9 ± 21.3 mg/kg d.m. for sparrowhawks to 279 ± 218 mg/kg d.m for mute swans. One of the mute swan specimens accumulated as much as 514 mg/kg d.m. of zinc in its liver.

Analyses of Cu levels in the livers of the examined birds showed that they accumulated, on average, from 9.6 mg/kg d.m. of this element (sparrowhawks) to 636 mg/kg d.m. (mute swans) (Table 2). For the latter species, particularly high levels of Cu were found in two specimens – as much as 893.7 and 1009 mg/kg d.m., respectively. Common buzzards, which specialize in catching rodents, accumulated Cu at an average level of 10.1 mg/kg d.m. A very similar level was found in the examined marsh harrier specimen (Table 1). In the examined common kestrels and long-eared owls, these values were slightly higher: 13.9 and 14.1 mg/kg d.m., respectively (Table 1).

The examined mute swans accumulated on average 7.3 mg/kg d.m. of manganese in their livers. Our study showed an interesting tendency for rodent-eaters from Eastern Poland, such as marsh harriers, common buzzards, long-eared owls and common kestrels, as well as piscivorous birds such as grey herons and goosanders, to accumulate Mn in a very narrow range of mean concentrations, respectively: 5.0 mg/kg d.m. and 6.5 mg/kg d.m. (Table 2).

*Toxic elements*

An important toxic element is lead. Concentrations of Pb between 0.37 and 3.87 mg/kg d.m. were found in the livers of all the examined birds from Eastern Poland (Table 2). The smallest of the obtained values referred to a grey heron individual and the highest concerned a rook specimen. The study revealed that, out of the birds examined, the highest mean concentrations of lead occurred in the livers of mute swans (Table 2).

During the study presented, cadmium was detected in the livers of all 41 birds. Only one rook individual exceeded the level (3.0 mg/kg d.m.) that might indicate increased environmental exposure. In the case of the second rook specimen, a noticeably increased concentration of Cd was found (2.38 mg/kg

Table 1. Concentration of lead (Pb), cadmium (Cd) and chromium (Cr) in liver [mg/kg dry mass] of individuals of studied birds. N – number of samples, TG – trophic guilds, SD – standard deviation, Min. – minimum value, Max. – maximum value, P – piscivorous, A – aquatic invertebrate eating, H – herbivorous, S – small mammal eating, B – bird eating, O – omnivorous, I – insectivorous, T – terrestrial invertebrate eating

| Species | N | TG | Zn | | Cu | | Mn | |
|---|---|---|---|---|---|---|---|---|
| | | | Mean±SD | min – max | Mean±SD | min–max | Mean± SD | min-max |
| *Botaurus stellaris* | 1 | A | 124.89 | – | 16.91 | – | 3.23 | – |
| *Ardea cinerea* | 8 | P | 115.28±34.59 | 73.70-177.08 | 21.07±12.64 | 10.77-47.31 | 6.43±3.71 | 1.38-11.92 |
| *Ciconia nigra* | 3 | P | 85.19±16.86 | 66.44-99.11 | 12.96±0.24 | 12.79-13.13 | 3.46±1.15 | 2.79-4.79 |
| *Cygnus olor* | 3 | H | 279.38±217.92 | 83.11-514.29 | 635.94±549.16 | 5.32-1008.82 | 7.92±5.42 | 3.44-13.94 |
| *Mergus merganser* | 1 | P | 55.18 | – | 11.53 | – | 6.51 | – |
| *Circus aeruginosus* | 1 | S | 30.32 | – | 9.68 | – | 5.27 | – |
| *Accipiter gentilis* | 2 | B | 104.77±110.46 | 26.66-182.87 | 9.58±2.73 | 7.65-11.51 | 6.40±3.54 | 3.91-8.91 |
| *Accipiter nisus* | 2 | B | 53.87±21.32 | 38.79-68.94 | 14.95±5.25 | 11.23-18.66 | 11.58±10.01 | 4.5-18.66 |
| *Buteo buteo* | 10 | S | 121.51±191.74 | 35.38-663.87 | 10.13±3.36 | 6.52-17.17 | 5.30±2.59 | 2.46-10.17 |
| *Falco tinunnculus* | 1 | S | 48.70 | – | 13.86 | – | 5.04 | – |
| *Asio otus* | 2 | S | 55.42±7.47 | 50.14-60.70 | 14.10±7.34 | 8.91-19.29 | 4.89±0.10 | 4.82-4.96 |
| *Corvus frugilegus* | 7 | O | 124.92± 63.34 | 72.18-245.68 | 11.80±2.46 | 8.68-14.09 | 2.53±1.22 | 0.73-4.44 |

Table 2. Concentration of lead (Pb), cadmium (Cd) and chromium (Cr) in liver [mg/kg dry mass] of individuals of studied birds. N – number of samples, SD – standard deviation, Min. – minimum value, Max. – maximum value, ND – not detected, TG – trophic guilds, P – piscivorous, A – aquatic invertebrate eating, H – herbivorous, S – small mammal eating, B – bird eating, O – omnivorous, I – insectivorous, T – terrestrial invertebrate eating

| Species | N | TG | Pb | | Cd | | Cr | |
|---|---|---|---|---|---|---|---|---|
| | | | Mean±SD | min - max | Mean±SD | min-max | Mean±SD | min-max |
| *Botaurus stellaris* | 1 | A | 1.80 | – | 0.50 | – | 0.31 | – |
| *Ardea cinerea* | 8 | P | 1.95±0.88 | 0.37-3.52 | 0.45±0.16 | 0.23-0.59 | 1.19±0.49(1) | ND – 1.80 |
| *Ciconia nigra* | 3 | P | 2.09±0.52 | 1.49-2.41 | 0.77±0.26 | 0.56-1.13 | 0.38(2) | ND – 0.38 |
| *Cygnus olor* | 3 | H | 2.73±0.24 | 2.57-3.00 | 0.60±0.00 | 0.16-0.90 | 0.53±0.21 | 0.30-0.71 |
| *Mergus merganser* | 1 | P | 1.36 | – | 0.87 | – | 0.68 | – |
| *Circus aeruginosus* | 1 | S | 2.77 | – | 0.82 | – | ND | – |
| *Accipiter gentilis* | 2 | B | 1.59±0.53 | 1.21-1.96 | 0.39±0.07 | 0.32-0.47 | 0.88 | ND – 0.88 |
| *Accipiter nisus* | 2 | B | 2.07±0.37 | 1.81-2.33 | 0.47±0.07 | 0.39-0.54 | 0.72 | ND – 0.72 |
| *Buteo buteo* | 10 | S | 1.98±0.48 | 1.48-3.05 | 1.01±0.51 | 0.48-2.36 | 0.55±0.28(3) | ND – 0.85 |
| *Falco tinunnculus* | 1 | S | 1.14 | – | 0.24 | – | 1.77 | – |
| *Asio otus* | 2 | S | 2.00±0.21 | 1.84-2.15 | 0.58±0.12 | 0.46-0.70 | ND | – |
| *Corvus frugilegus* | 7 | O | 2.35±0.88 | 1.36-3.87 | 1.98±0.72 | 1.12-3.50 | 0.87±0.31(4) | ND – 1.17 |

(1) n=5 specimens, (2) n=1 specimen, (3) n=3 specimens, (4) n=5 specimens

d.m.). Considering all the species examined, we can conclude that the highest concentrations of cadmium was found in rooks and buzzards – that is, birds that explore terrestrial ecosystems. Compared to rooks, four times less cadmium was found in the liver of the piscivorous heron (Table 2).

Concentrations of total chromium below detection level were found in 22 (53.7%) of the examined individuals of birds. The highest mean and maximum values of chromium concentration were found for grey herons and rooks. The result obtained for the single kestrel individual is also noteworthy (Table 2).

## DISCUSSION

Taggart *et al*. (2009) indicate that the hepatic zinc concentration level of 525 mg/kg d.m. (i.e. about 150 mg/kg wet mass) can be regarded as indicative of poisoning. In our study, we encountered only one such case among common buzzards. Zn concentrations in the livers of other examined specimens of common buzzards clearly correspond to the results of liver analyses performed for birds of this species from the south of Italy by Naccari *et al*. (2009) and Licata *et al*. (2011). These authors obtained Zn concentration values of 121.5 mg/kg d.m. and 137.5 mg/kg d.m. One of the mute swan individuals we examined accumulated an amount of zinc that was close to the level indicative of poisoning with this element (Table 1). Still, it is necessary to remember that researchers do not entirely agree on what level of Zn in the livers of birds indicates poisoning. Undoubtedly, interspecific differences are observed in water birds regarding the amount of zinc accumulated without physiological consequences. For example, Carpenter *et al*. (2004) report a case of a trumpeter swan *Cygnus buccinators* that appeared to have died of Zn poisoning and had 154 mg/kg wet mass of that element in its liver. In mallards *Anas platyrhynchos*, signs of poisoning were observed at Zn concentrations as low as 473 and 280 mg/kg d.m. (Levengoode *et al*., 1999; Sileo *et al*., 2003).

It has been reported that wetland birds can accumulate much larger amounts of copper than terrestrial birds (Horai *et al*., 2007; Schummer *et al*., 2011). Horai *et al*. (2007) found concentrations of copper as high as 4970 and 2420 mg/kg d.m. in the livers of grey heron and intermediate egret *Mesophoyx intermedia*, respectively, whereas the examined grey herons from Eastern Poland accumulated nearly 240 times less copper (Table 1). Such high levels of hepatic copper in mute swans can be logically explained by the fact that swans consume up to 35-43% of their body mass in aquatic vegetation daily (Willey and Halla, 1972). Copper is an essential micronutrient for all higher plants and is easily adsorbed by aquatic vegetation (Xue *et al*., 2010). Such high consumption of water plant biomass may contribute to the high levels of Cu in the livers of mute swans, which were found in two of the examined specimens. The result obtained for the third individual (given as the lower limit in Table 2) differed from the other two values, which resulted from starvation of this individual. Other researchers, who examined mute swans from northern Europe and North America (Clausem and Wolstrup, 1978; Schummer *et al*., 2011), have also reported concentrations of Cu comparable to those that we found in two of the examined birds. It is worth noting that the 2 specimens of mute swans mentioned here were in very good condition and were killed as a result of hitting electric tractions. On the other hand, it should be noted that even lower concentrations of copper (187-323 mg/kg) may be lethal for other wetland birds, such as Canada goose *Branta canadensis* (Henderson and Winterfield, 1975). We found considerably lower levels of copper among other birds we examined (birds of prey for instance), whose food did not include water vegetation. Hepatic copper concentrations in this case were many times lower and corresponded to the results obtained by other researchers (Jager *et al*., 1996; Horai *et al*., 2007; Zaccaroni *et al*., 2008). In the case of our measurements, the concentrations of copper in the livers of buzzards and kestrels were similar to the results obtained by other authors (Jager *et al*., 1996; Zaccaroni *et al*., 2008). It must be noted, however, that Licata *et al*. (2010), providing data from Sicily, gave values of hepatic copper concentration for buzzards (37.8 mg/kg d.m.) four times higher compared to results from Eastern Poland.

The amounts of manganese accumulated in the livers of mute swans turned out to be similar to the results presented by Schummer *et al*. (2011), who examined mute swans from the area of the Great Lakes of North America. For four groups of swans examined, between ten and twenty specimens each, they found the following hepatic manganese concentrations, 7.77, 7.85, 8.00 and 10.59 mg/kg d.m., respectively. For other species of wetland birds, hepatic manganese concentrations ranged from 3 to 11 mg/kg d.m. (Hui *et al*., 1998; Burger and Gochfeld, 1999; 2000). The rodent-eaters and piscivorous birds accumulated manganese in a very narrow range of concentrations, around 5 and 6.5 mg/kg d.m., whereas the studies of other authors show hepatic concentrations approximately 2 times higher compared to those we found. For example, Licata *et al*. (2010) established hepatic manganese concentrations at 9.0 mg/kg d.m. for buzzards from Sicily, and Horai *et al*. (2007) found an average of 13.4 mg/kg d.m. of manganese in the livers of grey herons from Japan.

Lead concentrations exceeding 6 mg/kg d.m. in the livers of birds may be regarded as causing sublethal or lethal toxicity (Pain and Amiard-Triquet, 1993; Battaglia *et al*., 2005; Martin *et al*., 2008). Fortunately, none of the birds examined showed hepatic lead concentrations above the considered threshold value (6 mg/kg d.m.). It is assumed that wild Mute swans apparently die from lead poisoning with as little as 10 to 14 mg/kg d.m. (approximately 3 to 4 mg/kg wet mass) in livers (Birkhead, 1982; Spray and Milne, 1988). Earlier studies demonstrated that lead is also accumulated in eggshells of birds such as the Mute swan and the Marsh harrier (Komosa *et al*., 2007).

It is obvious that the main source of lead in the case of wetland birds and raptors is the lead shot used by hunters (Martinez Haro *et al.,* 2002; Mateo, 2009). In Poland the use of lead shot by hunters and of lead sinkers by anglers is not prohibited. This results in high concentrations of lead in the bones and livers of ducks. For example, among Greater scaups *Aythya marila* and Common pochards *Aythya ferina* from the Szczecin Lagoon (NW Poland), as many as 25% and 46%, respectively, had over 10 mg/kg of lead in bones (Kalisiñska *et al*., 2007). As regards Mallards from the Szczecin Lagoon and the Sloñsk Reserve (NW Poland), 5.7% and 13.5% of shot birds, respectively, had more than

1.5 mg/kg of lead in liver wet mass (Kalisiñska *et al*., 2004). These birds often do not distinguish in the benthos pieces of lead from stones or shells when foraging and thus ingest them (Kalisiñska *et al*., 2004).

It is known that cadmium levels above 3.0 mg/kg d.m. in the liver might indicate increased environmental exposure (Scheuhammer, 1987; Burgat, 1990). Compared to other piscivorous birds, the examined herons accumulated less Cd. Studies on cormorants in Japan show that individuals from the vicinity of Tokyo accumulated 0.28 mg/kg d.m. on average, and those from the region of Lake Biwa 1.25 mg/kg d.m. (Saeki *et al*., 2000). As regards the grey herons examined in this study, they accumulated over two times less Cd (0.19 mg/kg d.m. on average).

Studies on chromium migration in the environment indicate the significance of river ecosystems in the transport of chromium ions and demonstrate the accumulation of this element in river sediments (Wong *et al*., 2002; Wong *et al*., 2007). The above allows us to understand why the highest mean and maximum levels of chromium concentration were found in birds very often foraging in rivers, as well as in the meadows and floodlands of river valleys. These birds were: grey heron, rook, and common kestrel. For 31 specimens of grey herons examined in Japan, mean concentrations of chromium were found to be 0.386 (ranging from 0.130 to 0.852 mg/kg d.m.) (Horai *et al*., 2007). That was approximately two times less compared to the results for grey herons from Poland. The maximum value of Cr concentration for Japanese grey herons was very close to the median value obtained for Polish specimens of the same species (0.863 mg/kg d.m.). Unfortunately, studies using atomic absorption spectroscopy do not allow to identify the ionic form of chromium. That is of particular importance in the case of this element, since Cr(VI) is strongly toxic while Cr(III) is an essential trace element (Levina *et al*., 2003).

Poland is one of Europe's leaders in cadmium and lead emissions. In 2007, the total emission of Cd and Pb in Poland was estimated at approximately 40 and 573 tons, respectively (Debski *et al*., 2009). An entirely new problem at present is electronic wastes, being a considerable source not only of Cd and Pb but also of Cr (Li *et al*., 2008; Robinson, 2009). Unfortunately we can not identify e-waste sources in Eastern Poland.

This problem was reflected in two EU Directives concerning the limitation of the amount of heavy metals (including Pb, Cd and Cr) in electronic products (RoHS 2003) and in electronic wastes (WEEE 2003). E-wastes are a fast-growing waste stream in Poland. Many Polish towns are facing problems with huge amounts of e-waste because rapid changes in computer technology encourage users to throw away old equipment. Colour cathode ray tubes (CRTs), obsolete computers and other electronic appliances form electronic waste. These e-waste contains, among others, hazardous substances, such as lead, cadmium, chromium, etc. It is also necessary to note the especially large amounts of Zn and Cu are introduced into the environment with e-waste (Robinson, 2009). In Poland, to all intents and purposes, the e-wastes storage and recycling system is only under construction (Biegañska and Szmigiel, 2010) and garbage of this kind ends up in standard dumps. The results of our study confirm this problem: we found the highest hepatic concentration of cadmium in a rook specimen. This can obviously be related to e-waste, since individuals of this omnivorous species are very often present in great numbers at large and local dumpsites.

## CONCLUSIONS

The study demonstrates that the concentrations of biogenic elements did not exceed levels indicative of poisoning (except in the case of one individual). Admittedly, the concentrations of lead detected in mute swans do not prove their poisoning, but they do indicate that lead shot remains a hazard to water ecosystems. Lead, cadmium and chromium concentration levels in the livers of omnivorous and piscivorous species point to the presence of these elements in the environment and testify to the increasing role of the so-called e-waste in the total amount of heavy metal contamination.

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## KOLIČINE ZNAČAJNIH (Zn, Cu, Mn) I TOKSIČNIH (Cd, Pb, Cr) ELEMENATA U JETRI PTICA IZ ISTOČNE POLJSKE

KOMOSA A, KITOWSKI I i KOMOSA Z

SADRŽAJ

Naša ispitivanja su bila fokusirana na određivanje koncentracije značajnih teških metala (Zn, Cu i Mn) i toksičnih metala (Pb, Cd i Cr) u jetri ptica iz istočne Poljske. Najveća prosečna količina cinka 279 mg/kg suve mase tkiva je izmerena kod nemih labudova. Samo kod jedne vrste analiziranih mišara koncentracija cinka je iznosila 664 mg/kg suve mase, što je ukazivalo na trovanje ovim elementom. Ptice koje se hrane glodarima su akumulirale mangan u jetri u uskom opsegu koncentracija u blizini prosečne vrednosti od 5,0 mg/kg suve mase. Opseg srednjih koncentracija mangana (oko 6,5 mg/kg suve mase) je takođe potvrđen kod ptica koje se hrane ribom. Najviše srednje vrednosti za olovo su izmerene kod nemih labudova (2,7 mg/kg suve mase), a kadmijuma kod vrana (2,0 mg/kg suve mase). Koncentracije ukupnog hroma iznad nivoa detekcije su pronađene kod 22 uzorka (53,7%), a vrednosti su bile najveće kod vrana. Rezultati naših analiza ukazuju da koncentracije biogenih elemenata nisu premašile nivoe koji ukazuju na trovanje (osim kod jednog uzorka). Ovo ispitivanje je dokazalo da olovna sačma ostaje opasnost u vodenim ekosistemima. Nivo olova, kadmijuma i hroma u jetri ptica svaštojeda i ptica koje se hrane ribom, ukazuje na stalno prisustvo ovih elemenata u okolini. To potvrđuje tezu o rastućoj ulozi elektronskog otpada, uključujući metalni elektronski otpad u ukupnoj kontaminaciji životne sredine.