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*Original article*

# Concentration levels of cadmium and lead in the raw and processed meat of *Helix pomatia* snails

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## Abstract

This work reports on cadmium and lead contaminations in the edible snail *Helix pomatia* harvested in Poland. One hundred and 24 samples of *Helix pomatia* meat collected from seven provinces (voivodeships) of Poland were analyzed for their trace metal levels by graphite furnace atomic absorption spectrometry (GFAAS). The research was conducted in 2 stages. The 1st stage analyzed snail meat prior to any further technological treatment (raw meat). In the 2nd stage, the trace element levels were measured in meat subjected to technological treatment (processed meat). The trace element contents in raw meat samples ranged from 0.06 mg kg$^{-1}$ to 0.22 mg kg$^{-1}$ for Cd and from 0.06 mg kg$^{-1}$ to 0.18 mg kg$^{-1}$ for Pb. The analyses revealed an increase in the cadmium content from 0.12 mg kg$^{-1}$ to 0.18 mg kg$^{-1}$ in thermally treated snail meat and no changes in lead concentration during the two-stage heat treatment. Regulation (EC) 1881/2006 does not specify the Cd and Pb residue limits in meat of terrestrial edible snails. The limits are set for invertebrate aquatic organisms meat (i.e. shellfish, mollusc, cephalopod) and range from 0.5 mg/kg to 1.5 mg/kg of tissue fresh weight for Pb and from 0.5 mg kg $^{-1}$ to 1 mg kg$^{-1}$ for Cd (EU Commission 2006). The results demonstrate that the land snail *Helix pomatia* has a tendency to bioaccumulate trace elements, and the cooking process is likely to affect (increase) the Cd content in the snail meat.

**Key words:** cadmium, lead, GFAAS, snail meat, Helix pomatia

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## Introduction

The rapidly growing sector of food production in the world has promoted the development and marketing of new types of food. The emergence of new food products results from the search for alternatives to animal-based protein sources and from endeavours to satisfy increasingly sophisticated tastes of consumers. Edible snail meat answers both these requirements. Currently, the most commonly consumed snail species in the world belong to the Achatinidae and Helicidae families, the former of which are eaten mainly in Africa, and the latter in Europe, especially France. In that country, snail meat is regarded as a sophisticated entry dish, but it is frequently served as a main course as well. Commercially available meat of edible snails comes from heliciculture farms, where mainly *Cornu aspersa aspersa* (CAA) and *Cornu aspersa maxima* (CAM) snails are bred, and from the free-living population of *Helix pomatia* (HP), called the Roman snail. Roman snails are harvested in their natural habitat, which has a considerable effect on the presence of toxic chemical residues, such as organophosphorus and organochlorine compounds and heavy metals, in their tissues (Coeurdassier et al. 2001). Several studies found that the levels of bio-accumulated trace metals in snails were highest in the hepatopancreas gland, and lower in the crop, kidney and foot (Coughtrey and Martin 1976, Coughtrey and Martin 1977, Dallinger and Wieser 1984, Hödl et al. 2010, Mleiki et al. 2016). These elements are also deposited in the shells (Aleksander-Kwaterczak and Gołas-Siarzewska 2015). The accumulation of heavy metals with the potential to transfer to higher trophic levels is of the uttermost importance in the terrestrial food chain and presents a risk to human health due to their toxicity. Exceeding the safe limits of human exposure to cadmium (Cd) and lead (Pb) may result in adverse effects at the physiological level, such as deposition in tissues, reproduction disturbances or nervous and hormonal disorders, as well as at the biochemical level, such as changes in DNA, mutagenic and carcinogenic effects or inhibition of protein synthesis and enzyme activity (Fowler 2009).

As far as heavy metals are concerned, the main threat to human health is posed by cadmium and lead, which are cumulative poisons with long biological half-lives and chronic toxicity. Regulation (EC) 1881/2006, setting the maximum levels of certain contaminants in foodstuffs, does not specify the Cd and Pb residue limits in meat of terrestrial edible snails. The maximum regulatory levels set for invertebrate meat (i.e. shellfish, mollusc, cephalopod) range from 0.5 mg/kg to 1.5 mg/kg of tissue fresh weight for Pb and from 0.5 mg $kg^{-1}$ to 1 mg $kg^{-1}$ for Cd (EU Commission 2006). However, these limits apply only to aquatic organisms and not to land snails. According to the EU specific rules on the hygiene of food of animal origin in section XI of Regulation EC 853/2004, snail meat intended for human consumption must be prepared and handled exclusively in approved establishments constructed, laid out and equipped for that purpose. Then, the raw material obtained must be subjected to organoleptic examination and, in the case of snails, the hepatopancreas must be removed if it presents a hazard (EU Council 2004). Roman snails can be transported alive and hibernated or in the form of frozen meat, which is obtained by dividing their carcasses into edible and digestive portions. The edible portion includes the foot with the collar and a fragment of the mantle, whereas the digestive portion consists of the visceral sac with the midgut gland and other internal organs.

The aim of the present study was to compare Cd and Pb contents in the meat of Roman snails from seven provinces of Poland. In addition, the effect of preliminary technological treatment of snail meat on the concentration of these trace elements was assessed. The research presented here was the first to determine Cd and Pb levels in the edible portion of free-living *Helix pomatia* snails collected in Poland. It is of considerable importance because of the increasing demand for snail meat, especially from Central and Eastern Europe. Snails are exported alive and hibernated or in the form of frozen or processed meat.

## Materials and Methods

### Sampling procedure

The research material comprised 124 samples of the meat of Roman snails harvested in seven provinces (voivodeships) of Poland: West Pomerania (12 samples), Lubusz (12), Greater Poland (20), Lower Silesia (20), Opole (20), Warmia-Masuria (20) and Podlasie (20). The snails were obtained in accordance with the Regulation of the Ministry of Environment on the protection of animal species (ISAP, 2016). In Poland, the harvest season for Roman snails for the food processing industry is strictly defined (i.e. from April 20 until May 31), and it is required that the snail shell diameter should be over 30 mm. In addition, snails can be collected solely in designated areas, in amounts limited by the Regional Directorates for Environmental Protection (ISAP, 2016). The snails under study were obtained from snail-purchasing centres operating in the aforementioned provinces. Snails with a shell diameter above 30 mm and body weight over 15 g bought from different locations in a province were randomly selected to form a 1-kg sample. The snails were sacrificed

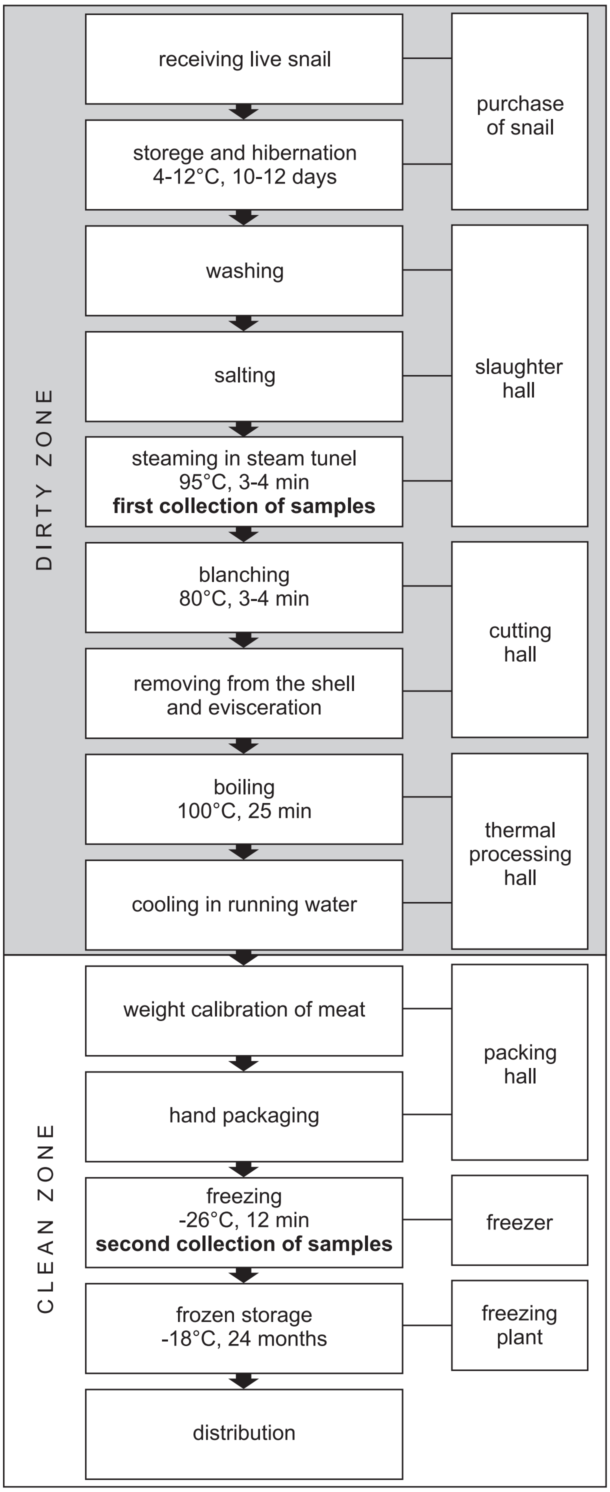

Fig.1. The pattern of snail meat processing stages.

according to an approved technological procedure in a food-processing plan (Fig.1).

## Sample preparation and examination

The research was conducted in 2 stages. In the 1st stage, determinations were performed on snail meat before any further technological treatment (raw meat), immediately after the snails were sacrificed. In the 2nd stage, determinations were made on frozen meat after technological treatment in an approved food-processing unit. The meat was thawed in a refrigerator at 4°C for 24 hours prior to the planned examination. The study involved the edible portions of snails, that is, the foot with the collar and a fragment of the mantle. A laboratory sample was composed of 20 snail carcasses homogenized with a hand blender to ensure the uniformity of research material. Then a 0.5-g analytical sample was produced from the homogenate and subjected to acid mineralization with 3 ml $HNO_3$ in a Mars Xpress microwave mineralizer (CEM, USA). Three degrees of mineralization were applied. The samples were allowed to ramp to 90°C for 15 min and digest at 90°C for 5 min, ramp to 120°C for 10 min and digest at 120°C for 10 min, ramp to 210°C for 15 min and digest at 210°C for 20 min. Subsequently, the sample liquid was poured into volumetric flasks, and demineralized water was added to a level of 50 ml. Cadmium and Pb levels were determined by graphite furnace atomic absorption spectrometry (GFAAS) in a SpectrAA 280Z atomic absorption spectrometer (Varian, Australia). Instrumental parameters for GFAAS were set as follows: 228.8 nm wavelength, 0.5 nm slit width, 4 mA lamp current for cadmium, and 283.3 wavelength, 0.5 nm slit width, 10 mA lamp current for lead (Table 1). The analysis used the AccuTrace reference standards of 99.9 % purity.

Table 1. Instrumental parameters for GFAAS determination.

| Parameter | Cd | Pb |
|---|---|---|
| Wavelength (nm) | 228.8 | 283.3 |
| Slit (nm) | 0.5 | 0.5 |
| Measurement time (s) | 3 | 3 |
| Lamp current (mA) | 4 | 10 |
| Atomization (°C) | 1800 | 2300 |

## Statistical analysis

The results were analyzed statistically on the basis of arithmetic means and standard deviations. The analysis included a comparison of heavy metal contents (Cd and Pb) in snail meat subjected to the two-stage technological processing, followed by a comparison of these trace element contents in samples from the 7 provinces. A normal distribution in each group was checked by the Shapiro-Wilk test. The effect of variability factors on the parameters analyzed was established on the basis of a one-way analysis of variance (ANOVA) at the 5% significance level. In the case of the rejected null hypothesis that all the means are

Table 2. Impact of harvest location and meat technological treatment on Cd level in *Helix pomatia* (HP) meat (mg kg $^{-1}$).

| Voivodeships | Raw meat | | | Processed Meat | | |
|---|---|---|---|---|---|---|
| | Mean | SD | Min-max | Mean | SD | Min-max |
| West Pomeranian | 0.22 aD | 0.04 | 0.16-0.26 | 0.34 bD | 0.09 | 0.29-0.44 |
| Lubusz | 0.13 aC | 0.02 | 0.12-0.15 | 0.17 bB | 0.03 | 0.15-0.19 |
| Greater Poland | 0.09 aB | 0.01 | 0.08-0.10 | 0.16 bB | 0.04 | 0.11-0.20 |
| Lower Silesian | 0.11 aC | 0.03 | 0.08-0.15 | 0.18 bB | 0.03 | 0.15-0.19 |
| Opolskie | 0.13 aC | 0.03 | 0.12-0.15 | 0.22 bC | 0.04 | 0.21-0.22 |
| Warmia-Masuria | 0.06 aA | 0.01 | 0.05-0.06 | 0.09 bA | 0.01 | 0.09-0.10 |
| Podlaskie | 0.07 aA | 0.02 | 0.05-0.09 | 0.12 bA | 0.04 | 0.07-0.16 |
| Total | 0.12 a | 0.05 | 0.05-0.26 | 0.18 b | 0.08 | 0.07-0.44 |

A, B, C – the mean values marked with capital letters differ statistically significantly at $p \leq 0.05$ vertically
a, b – the mean values marked with small letters differ statistically significantly at $p \leq 0.05$ horizontally

Table 3. Impact of harvest location and meat technological treatment on Pb level in *Helix pomatia* (HP) meat (mg kg $^{-1}$).

| Voivodeships | Raw meat | | | Processed Meat | | |
|---|---|---|---|---|---|---|
| | Mean | SD | Min-Max | Mean | SD | Min-Max |
| West Pomeranian | 0.09 B | 0.02 | 0.07-0.11 | 0.1 B | 0.03 | 0.07 -0.12 |
| Lubusz | 0.10 B | 0.01 | 0.07-0.11 | 0.10 B | 0.02 | 0.08-0.12 |
| Greater Poland | 0.18 C | 0.05 | 0.13-0.22 | 0.15 C | 0.05 | 0.09-0.23 |
| Lower Silesian | 0.08 B | 0.01 | 0.07-0.09 | 0.09 B | 0.05 | 0.04-0.17 |
| Opolskie | 0.12 B | 0.05 | 0.07-0.17 | 0.10 B | 0.01 | 0.09 -0.12 |
| Warmia-Masuria | 0.06 A | 0.03 | 0.04-0.07 | 0.05 A | 0.01 | 0.05-0.06 |
| Podlaskie | 0.06 A | 0.02 | 0.02-0.03 | 0.04 A | 0.02 | 0.03 -0.07 |
| Total | 0.10 | 0.06 | 0.02-0.22 | 0.08 | 0.05 | 0.03-0.23 |

A, B, C – the mean values marked with capital letters differ statistically significantly at $p \leq 0.05$ vertically

equal, a multiple-comparison post-hoc test with Tukey confidence intervals was applied to establish which groups differed statistically significantly from each other. The significance of differences between the features under study was determined at a level of $p \leq 0.05$. The computations were made with the Statistica 10 software package (StatSoft).

## Results

### Effect of snail harvest location on Cd and Pb levels in snail edible portion

The effect of the snail harvest location and the preliminary technological treatment on Cd and Pb concentrations in the edible portion of Roman snails is presented in tables. (Tables 2 and 3).

It was found that the snail collection site had a major impact on Cd and lead contents in meat. Significant differences in Cd and Pb concentrations were revealed between the raw meat of snails obtained from different provinces, and these differences were confirmed in the meat after technological processing. The lowest Cd level was established in the raw meat of snails from Warmia-Masuria and Podlasie Provinces, that is, 0.06 and 0.07 mg kg$^{-1}$ of meat, respectively. A slightly higher cadmium content (0.09 mg kg$^{-1}$) was determined in raw meat from Greater Poland Province, and higher still in raw meat samples from Lubusz (0.13 mg kg$^{-1}$), Lower Silesia (0.11 mg kg$^{-1}$) and Opole (0.13 mg kg$^{-1}$) Provinces. The highest Cd concentration, amounting to 0.22 mg kg$^{-1}$, was detected in the meat of snails collected in West Pomerania Province. With regard to lead, its lowest level in raw meat was also established in Podlasie and Warmia-Masuria Provinces (0.06 mg kg$^{-1}$). It was significantly higher in West Pomerania (0.09 mg kg$^{-1}$), Lubusz (0.1 mg kg$^{-1}$), Lower Silesia (0.08 mg kg$^{-1}$), and Opole (0.12 mg kg$^{-1}$) Provinces, and the highest in Greater Poland Province (0.18 mg kg $^{-1}$).

### Impact of technological treatment of meat on Cd and Pb levels

The present study revelated that technological processing influenced the cadmium level in the edible portion of snails. The cadmium concentration was significantly higher in thermally treated meat. This finding held true for snail meat from all 7 provinces. With regard to the Pb content, no statistical differences were observed between cooked and raw meat from any of the provinces.

## Discussion

### Effect of *Helix pomatia* harvest location on Cd and Pb levels

The analyses performed clearly demonstrate that free-living land snails of the *Helix* genus are likely to be a source of exposure to heavy metals in human diet. The presence of Cd and Pb in the tissues of *Helix pomatia* snails results, among others, from their ability to assimilate and accumulate these heavy metals. It has been confirmed that Roman snails are able to accumulate heavy metals from both polluted and relatively clean environments (Gomot de Vaufleury and Pihan 2000). Studies on Cd and Pb accumulation by HP snails demonstrate that snails of this genus are macroconcentrators for Cd and microconcentrators for Pb, and their hepatopancreas is where these elements accumulate (Dallinger and Rainbow 1993, Menta and Parisi 2001). The available data show that digestive gland tissues of the Roman snail can accumulate as much as 90% of Pb and 55%-70% of Cd, whereas the rest of the assimilated Cd and Pb is deposited in the foot and shell (Dallinger and Wieser 1984, Nowakowska et al. 2012, Nica et al. 2014, Aleksander-Kwaterczak and Gołas-Siarzewska 2015). With regard to Pb, it has been found that the well-developed and highly effective regulatory mechanisms for this element prevent its excessive accumulation in snail tissues (Bebby 1985). It is worth noting that snails play a very important role in transferring toxic elements to higher trophic levels of terrestrial food chains. Notably, the snail's ability to transfer Cd and Pb in the soil-plant-snail food chain is considered extremely dangerous (Notten et al. 2005, Nica et al. 2012), as it poses the risk of human intoxication. The research results demonstrate that the high Cd content in the snail edible portion may be closely related to the elevated concentration of this element in the topsoil of the areas where the snails have been collected (Nica et al. 2015). In Poland, increased amounts of Cd in soil are reported in industrial areas of Upper and Lower Silesia (1-5 mg $kg^{-1}$) and in the vicinity of large urban agglomerations (up to 41 mg $kg^{-1}$), which is associated with anthropogenic activities (Maciołek et al. 2013). This is evidenced by the high Cd residue levels in the meat of snails harvested in Lower Silesia, Opole and Lubusz Provinces. The high cadmium levels in the Oder valley (in West Pomerania Province), which locally exceed 4 mg $kg^{-1}$, are an anomaly compared with the generally low cadmium content in the surrounding region. Cadmium contamination in this area is attributed to the local organic soils which favour accumulation of the element carried by the polluted Oder River from southern Poland. West Pomeranian Province had the highest Cd content among the seven provinces studied. The regions with soils containing the lowest Cd concentrations in Greater Poland, Warmia-Masuria and Podlasie Provinces in central and eastern Poland were also characterized by a lower Cd content in the snail meat. Cd residue levels in HP snail meat correlate with the Cd content in soils, which confirms the ability of HP snails to assimilate and accumulate this element.

With regard to Pb, its highest concentration (above 50 mg $kg^{-1}$) was found in the soils of Upper and Lower Silesia (Lower Silesia and Lubusz Provinces). It is related to the occurrence of Triassic metalliferous dolomites and to mining-metallurgical activity (Polish Geological Institute). Pb levels in other regions of Poland are lower. The lead content in soil is the lowest in the eastern part of Poland (Warmia-Masuria and Podlasie Provinces), where it does not exceed 13 mg $kg^{-1}$. It is higher in the western part of the country (West Pomerania and Lower Silesia Provinces), ranging between 9 and 25 mg $kg^{-1}$. A higher Pb concentration was also found in the western part of the Polish Lowlands, as compared to the eastern part. The results of Pb level assessment performed in this study correspond with the geochemical analysis of the topsoil by the Polish Geological Institute (Polish Geological Institute). The comparison of heavy metal contents in the meat of terrestrial snails of the Helicidae family intended for food industry showed that Cd concentrations in the meat of free-living Roman snails and *Helix aspersa maxima* snails farmed in Poland are similar, with an average of 0.35 mg $kg^{-1}$ (Cicero et al. 2015). With regard to *Helix aspersa*, *Helix pomatia* and *Helix aperta* snails farmed in Italy, their cadmium concentration ranged between 0.08 and 4.01 mg $kg^{-1}$ (Corda et al. 2014). The Cd level in free-living HP snails harvested in Romania was higher than in Poland, ranging from 0.13 to as high as 3.12 mg $kg^{-1}$ (Toader-Williams and Golubkina 2009). As far as Pb is concerned, its concentration in *Helix aspersa maxima* snails obtained in Poland averaged 0.05 mg $kg^{-1}$, whereas in those farmed in Italy it ranged from 0.05 up to 1.12 mg $kg^{-1}$. Notably, some HP snails collected in Romania had a lead level as high as 48.08 mg $kg^{-1}$. In another species of edible snails, namely *Helix aspersa aspersa* in Greece, the levels of both Cd and Pb were below the limit of detection (Cicero et al. 2015).

### Effect of thermal treatment on Cd and Pb levels in edible snail meat

The available literature commonly states that the cooking process is likely to affect Cd and Pb contents in meat, causing an increase or decrease in the concentration of these heavy metals (Ganjavi et al. 2010). It has been established that the content of these elements in processed meat depends on a number of factors, including the meat type, duration and temperature of heat processing and the medium of cooking (Hoha et al. 2014). The present study revealed an increased cadmium content in thermally treated snail meat as compared with its level in raw meat. A similar increase was found in the meat of brown crab (*Cancer pagurus*), Chilean blue mussel *(Mytilus chilensis)* and sea bass fillets (*Dicentrarchus labrax*). (Ersoy et al. 2006, Ersoy 2011, Houlbréque et al. 2011, Talab et al. 2014, Wiech et al. 2017). The elevated Cd content can be linked to water loss in the protein denaturation process, which increases Cd concentration in the tissue. In case of snail meat, a secondary contamination of the edible portion by Cd accumulated in the hepatopancreas can also play a significant role. The secondary contamination process is of particular importance for small-sized snails, which can be ingested whole, without removal of the midgut gland prior to heat treatment, or when the division into the edible and digestive portions has disturbed the structure of the gland. Cadmium is bound in the cytoplasm of hepatopancreatic digestive cells by low-molecular weight proteins, metallothioneins. These proteins are characterized by a high content of cysteine, in which sulphur atoms of sulphurhydrogen groups form thermolabile Cd-SH bonds. Cadmium released during the cooking process can be a source of a secondary contamination of uncontaminated meat. A similar phenomenon has been described in the thermal processing of crabs (Włostowski et al. 2016, Wiech et al. 2017).

No changes in Pb concentration were observed during the two-stage heat treatment. Similar findings have also been reported for fish fillet meat subjected to thermal processing and obtained from bream *(Abramis brama)*, carp *(Carrassiusus auratus)*, perch *(Perca fluviatilis)* and rapacious carp *(Aspius aspius)* (Diaconescu et al. 2013, Heque et al. 2014). Roman snails possess a more efficient system for the elimination of excessive lead by mucous cells of the posterior section of the alimentary tract. The occurrence of Pb in the intestinal mucous cells may be attributed to a high content of sulphur amino acids, which are an important constituent of the mucus and bind Pb ions. During the scarification process with water vapor, snails excrete large amounts of mucus, which leads to Pb reduction in tissues. This initial Pb reduction process may explain the lack of significant differences in Pb concentration between the raw meat and meat subjected to thermal treatment.

## Conclusion

The present results show that free-living land snails of the Helix genus can be a source of heavy metals in human diets and the snail collection site can be an important factor influencing on Cd and Pb contents in their edible tissues. The maximum levels of Cd and Pb in terrestrial snail meat are not set in regulation (EC) 1881/2006.

Technological processing can also influence cadmium level in the edible portion of snail. The Cd concentration in the thermally treated meat was significantly higher than in the raw meat.

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