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Predicted intake of trace elements and minerals via household drinking water by 6-year-old children from Krakow (Poland). Part 5: Zinc

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6 **year-old children from Krakow (Poland). Part 5: Zinc**
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Abstract

Zinc(Zn) exposure in pre-school children via household drinking water collected by double sampling method (morning, evening) was evaluated in a sample of the Polish population. The Zn concentration was measured by the Flame Atomic Absorption Spectrometry. Rural and sub-urban Krakow sites were non-distinguishable in respect of Zn concentrations. However, significantly lower Zn was found in urban as compared to non-urban sites, (geometric mean(95% confidence interval) 0.14 (0.01-1.95) mg L⁻¹ vs. 0.52 (0.03-10.2) mg L⁻¹, p<0.001. Zn levels in water standing overnight in pipelines were higher in all sites by 0.36 mg L⁻¹ on average, but observed contaminations were higher. The Zn limit based on taste and colour of for drinking water (3 mg L⁻¹) was exceeded in 1 and 10% of households, from urban and non-urban sites, respectively. The Zn intake predictions for evening water samples for 6 years old children averaged between 2% and 9% of the Recommended Dietary Allowance (RDA, 10 mg day⁻¹) for urban and non-urban sites, respectively. Mean Zn intake prediction for the exceedance fraction was 64% of RDA. In conclusion, over-night contamination of drinking water from in-house pipelines was common to a significant and similar extent at all sites investigated. Secondly, drinking water can be a considered a significant contributor to dietary Zn intake by children in non-urban sites and may shift the population borderline of deficiency.

Keywords: *drinking water, zinc intake, water contamination, health protection, children*

Introduction

Multi-factorial implication of zinc (Zn) in human functioning results in complex aspects to be considered including nutritional adequacy, borderlines of malnutrition and overload, toxicity and numerous interferences (WHO 2003). There are several known beneficial but disadvantageous effects of zinc. Acute zinc toxicity is rare, and mild toxic events were mainly related to supplemental overload. Recent studies in focus suggest that Zn can impair cognitive functions and memory (Flinn et al. 2005) and seems to be involved in neuro-degeneration events (e.g. Zatta et al. 2009) together with copper and iron. Both, zinc overload as well as its deficiency can impair human health, suggesting that Zn balance is of a key importance. Steady supply, or its lack, from every-day dietary source, namely drinking water, is thus worth for closer look-up.

The major health concern of zinc is focussed on nutritional inadequacy of the population, since the population at risk of deficiency worldwide was estimated as high as 20.5% (Hotz et al. 2004, Fischer Walker et al. 2008). Zn absorption varies depending on diet and Zn content and reaches up to 92% at low Zn intakes (Hunt et al. 2008). Zn absorption is strongly associated with protein content in foods (Wapnir 2000), and insufficient protein intake may be the cause of zinc deficiency.

A recent study of populations in four EU countries revealed significant regional differences related to dietary profiles (Marcellini et al. 2006) and suggested a risk of Zn deficiency (Cruz 2000). Some *ad hoc* evaluation studies in Poland suggested a significant deficit of zinc in the Polish population (Stopnicka et al 1999). A study of women with low physical activity indicated Zn intake to be below recommended levels (Bronkowska and Karcz 2007). In the study in southern Poland a marginal zinc deficiency was identified in about 16% of 11-year-old children (Schlegel-Zawadzka et al. 2002). Similarly, significant risk of Zn deficiency was suggested in about a half of female population of Polish university students (Szymelfejnik et. al. 2008) and adolescents (Augustyniak and Brzozowska 2002).

DW was rarely included as explicit contributor to intake of nutrients. According to studies reviewed in the background WHO document on zinc in DW, tap-water can provide up to 10% of the daily intake (WHO 2003). In the study in Poland, DW was found to be a considerable source of zinc intake for elderly (Kaluza et al. 2002).

Besides, natural waters zinc, like copper, is as low as at microgram levels and can reach milligrams levels at tap, depending on corrosion of metallic supply systems. Corrosion-derived fractions of zinc and copper in DW originate from different parts in pipeline system. However, mixed iron-zinc-copper (and lead) pipeline systems are especially liable to electrochemical processes and dissolution of less noble metal.

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3 Zinc in drinking water at concentrations above about 3 mg L⁻¹ can give rise to problems with
4 appearance and taste to humans. Because of low Zn levels observed in DW and its organoleptic properties
5 at excess, the presence of Zn in drinking water was not regarded to be of human exposure concern. WHO
6 guideline value for Zn in DW was suggested at 3 mg L⁻¹, and 5 mg L⁻¹ by US EPA (2002), while no
7 regulation was established in EC (1998). In 2003 WHO agreed the Zn risk assessment to be brought
8 forward to the current edition of the *Guidelines for Drinking-Water Quality* (WHO 2006). Polish
9 regulation in force until 2002 allowed 3 mg Zn L⁻¹, then abstained from defining an allowable Zn limit
10 ((PMH 2007) following EC regulation.
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18 Based on former WHO recommendations, Dietary Allowance values suggested for Polish
19 population (RDA, safe level) ranged depending on age and gender between 5-21 mg day⁻¹ (Ziemiński et
20 al 1998). New Dietary Reference Intakes (DRI) for North Americans were set at 2-13 mg day⁻¹ and upper
21 intake levels was set at 40 mg day⁻¹ (US DRI 2000). An evaluation of methodological approaches of
22 current nutritional recommendations in EC countries has been published recently (Doets et al. 2008). As
23 one of the highest priority nutrients, zinc was scheduled for early updating actual Dietary Reference
24 Values (DRV) for EC (EFSA 2008). Zinc is a subsequent element in our studies (Barton 2009) analysed
25 in household drinking water and evaluated for extent of contamination in pipelines and for nutrient intake
26 predictions in 6-year-old children.
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36 **Materials and methods**

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38 The study area and population were described previously (Barton 2008). Briefly, the study comprised ca.
39 600 households and pre-schools and was performed in the period 1997-2004. From about a half of
40 households 5-7 year-old pre-school children were included. The drinking water (DW) samples were
41 collected in households by double sampling method, in the morning (W1 or DW1) and in the evening
42 (W2, DW2), in Kraków urban (U) and suburban (P) sites and rural (R) areas of south eastern Poland.
43 The anthropometrical data of children were previously published (Barton 2005). Characteristics of the
44 current group of children for Zn evaluation were (mean±standard deviation): N=314, gender structure
45 45.5% boys; age 6.6±0.6; body weight 24.1±4.5 kg; height 1.23±0.07 m; body mass index (BMI)
46 16.0±2.4 kg m⁻²; adequate water intake (AWI) 1.56±0.06 liter was calculated as described previously
47 (Barton 2002).
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58 *General methodology*

59 The analytical method fulfilled the best laboratory approaches for trace elemental analysis, as described
60 earlier (Barton et al. 2002). High purity deionised water (Milli-Ro & Milli-Q, Millipore), reagents and

standards for calibration and rigorous quality control of analyses were applied. All measurements were done in 0.5% (v/v) nitric acid.

Analytical characterisation

Zinc analyses were performed by means of flame atomic absorption spectrometry (F-AAS, Perkin-Elmer 5100ZL) at parameters: 10 cm long burner, hollow-cathode lamp (HCL), analytical wavelength 213.9 nm, slit 0.7 nm, flame gas air-acetylene flow rates ratio (in litre/minute) 10 : 2, injection flow rate ca. 1 ml/minute, analytical signal - 3 sec time averaged absorbance, measurement run - three successive signals, estimation - duplicate successive runs accepted if the relative standard deviation was below 5%.

The analytical characteristics were as follows: calibration range 0-2 mg L⁻¹, analytical limit of detection (LOD, 3SD) and quantitation (LOQ, 10SD) was obtained at 0.005 mg L⁻¹ and 0.017 mg L⁻¹ (N=27), respectively; the method LOD and LOQ was 0.013 mg L⁻¹ and 0.043 mg L⁻¹, respectively; precision for Zn standard 0.1 mg L⁻¹ was 2.6%, and 1.3-1.6% in the range 0.25-2.0 mg L⁻¹; recovery 97-102%.

Data processing

The non-linear curves were obtained within the calibration range. Sensitivity of analytical system was tracked during the analyses with control standard and blank measured every ten runs. Machine concentration estimations were tentative only and final results were obtained by more sophisticated procedure described previously. The software-collected internal signals were subjected to post-measurement processing consisting of: 1) precise re-calibration via estimation of analytical form of calibration equation, 2) estimation of numerical time(run)-functions with non-linear fitting, describing the signals of cyclic quality controls. The equations were further used for correction of the sensitivity drift and slow waving by interpolation and with combination of recalibration equations lead to final estimations, as described earlier (Barton 2008). All numerical processing were smoothly performed with home-made macros for commercial software.

Calculations and statistics

Concentrations below limit of detection were replaced for convenience by the ½ LOD values. The metal intakes were calculated from approximated adequate daily water requirement approximated by 1.5±0.25 litre, as described previously [Barton et al. 2002]. The relative intake was calculated as percentage of RDA values at safe level 10 mg day⁻¹ for 4-6 year-old Polish children (Ziemiński et al. 1998).

The cumulative uncertainty of the metal intake was approximated by the sum of particular

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3 relative contributions and adjusted to the coverage of 95%, obtaining the values 63% or 67% for intake
4 per kg b.wt.

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7 Relative indices of changes in metal concentration in water during standing in pipelines were
8 expressed as a combination of morning and evening concentrations (W1, W2): $R_{1,2}$ - ratio of W1:W2;
9 $D_{1,2}$ - difference of W1 - W2 and $D_{1,2\%}$ - as its percentage (relative difference) vs. higher metal
10 concentration, W1 or W2, obtaining two sets of values, positive (fraction 'a') - contamination and
11 negative (fraction 'b') - deposition.
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16 Statistical analysis was performed with the use of *Statistica for Windows* (StatSoft, inc.). Both
17 non-parametric and parametric tests were applied, if necessary. The t-test or Kruskal-Wallis ANOVA
18 and χ^2 (chi-square) were used, if were applicable. All mean values were geometric means (GM) or/and
19 medians (MED), if not stated otherwise. The 95% confidence interval (CI) was estimated from
20 distribution profile as $GM \cdot SDg^{\pm 1.96}$ or from frequencies as 2.5 and 97.5 percentiles.
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26 27 **Results**

28 29 30 *Zinc concentrations in drinking water*

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32 Among ca. 600 investigated households, the maximum zinc concentration in DW was 17.3 and 9.0 mg L⁻¹
33¹, for morning (W1) and evening (W2) series, respectively. No significant time-trend in Zn concentration
34 was observed comparing the period before and after year 2000 in any site and sample series. Peripheral
35 and rural sub-sites were comparable, thus these sites can be discussed as a uniform non-urban site (NU).
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40 Distribution of Zn concentrations in evening DW series for U and NU-sites is shown in Figure 1.
41

42
43 [Insert Figure 1 about here]

44
45 The frequency profiles were strict lognormal for both U and NU subsets. Number of analytical
46 outliers below LOD was marginal (<1%). The distribution for U sites was significantly different
47 ($p < 0.001$) and shifted down vs. NU concentrations. There is no visible indication of multi-modal structure
48 of the both profiles.
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53 Mean Zn concentrations in W1, W2 samples for U, P and R and combined sites are collected in
54 Table I.
55

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57 [Insert Table I about here]

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60 Zn concentration was substantially lower at U sites: GM in W2 samples (mg L⁻¹) were 0.14 while

0.52 for NU sites.

Strong differences were observed between W1 and W2 series in every site ($p < 0.001$). W1 concentrations were two-three times higher, as indicated $R_{1,2}$ values in Table I. The related parameters (difference and ratio) were calculated for the major fraction of households, where metal concentration increased after overnight stagnation in pipelines. The contamination was not site dependent, if absolute values ($D_{1,2}$) were compared for U vs. NU ($p > 0.05$). Relative contaminations ($D_{1,2\%}$ as $D_{1,2}$ per cent) or a ratio ($R_{1,2}$) were different. Mean relative increase of Zn in DW due to interior contamination was 66% and 49% for U and NU sites, respectively.

The exceedance of aesthetic limit of 3 mg L^{-1} was negligible in U sites and significant in NU sites (18-22% for W1 and twice less for W2).

Metal concentration in morning and evening samples

Distribution of metal concentrations in DW samples was shown as two-dimensional projection (evening vs. morning) in Figure 2. Each point represents one household for which a pair of samples was collected.

[Insert Figure 2 about here]

The two cut-off lines refer to the aesthetic Zn limit in drinking water in force in the period of sample collection, while line 1:1 refers to W1 and W2 equality. The sectors A - C comprise the households for which metal concentration fell below the limit for both W1 and W2 samples (A), over the morning or evening (B1 or B2) and over the limit for both (C). Inserted box in Figure 2 shows percentages of households in particular sectors.

Significant number of households, about 10% from NU sites, contained Zn over the aesthetic limit both in W1 and W2. The sum of percentages in respective sectors refers to the exceedance given in Table 1 at some approximation due to single values.

Metal concentrations in water from the major water treatment plants

Five major water treatment plants (WTP) supplying water to study areas in Kraków were shown on the map in Part 3 (Barton 2008). The largest area is supplied by soft water from the Raba River (A) and the other sources are local rivers (B-D) and a deep well (E).

Mean metal concentrations in household tap water (W1, W2) from the suppliers and percentage of contribution to the total water consumption are shown in Figure 3.

[Insert Figure 3 about]

Zn concentrations in DW from sources A-E were within the range (GM in mg L⁻¹) 0.37-0.97 and 0.16-0.42, in W1 and W2, respectively. The differences between sources were significant in both W1 (p<0.01) and W2 (p<0.05), and between pairs W1 and W2 in each group (p<0.05 at least).

Weighted mean metal concentration by contributions of five supply systems to water consumption, reflecting average Zn concentration in water potentially consumed by population of Krakow was 0.49 and 0.18 mg L⁻¹, for W1 and W2, respectively.

Influence of the pipelines

The effect of overnight stagnation in pipelines on the changes in metal concentration depending on its background concentration in W2 was shown by different projections in Figures 4A-B. W2 taken in the evening was assumed to be fully flushed water not influenced by pipeline interior and to correspond to metal concentrations in water supplied by the WTPs. The households were divided into two fractions, and those where Zn concentration increased were designated as 'a' (D_{1,2} > 0) or decreased, 'b' (D_{1,2} < 0). The plots show absolute values of concentration change (D_{1,2}), its percent (D_{1,2}%), and the ratio (R_{1,2}) of W1 to W2.

[Insert Figure 4A and 4B about here]

In ca. 80% households Zn concentration increased in over-night standing water due to contamination (mode 'a') and a decrease was observed for a remaining households ('b'). Both effects were accelerated with increase in background Zn (W2).

In the Figure 4A, in the mode 'a' a plateau at the average D_{1,2} value 0.70 mg L⁻¹ was observed in the middle range of Zn concentrations and comprised 49% of fraction 'a'. For fraction 'b' the percentage of deposition (Figure 4B) was practically independent of Zn in W2 and the average was ca. - 40% for most of 'b' samples.

The above plots drawn separately for U and NU domains albeit qualitatively similar show certain quantitative differences, what will be described elsewhere.

Zinc intake with drinking water by children

The intake predictions were calculated using adequate water intake (1.5 L day⁻¹) and anthropometric data of study group of children. The intakes were expressed as daily intakes, daily intakes per kilogram of body weight and as a percent of Polish RDA for 4-6 year old children, separately for both, W1 and W2 water. Two criteria were applied for selection of 'risk groups', i.e. exceedance of the Zn concentration aesthetic limit (risk group 1) and RDA for 4-6 year old children (risk group 2). Mean predicted Zn intakes from W1

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3 and W2 drinking water by 6-year-old children for selected subgroups were collected in Table II.
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6 [Insert Table II about here]
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9 Mean predicted Zn intakes for the whole group were (GM) 0.82 and 0.38 mg day⁻¹ for W1 and
10 W2, respectively, and contributed by 5.6-17.6 and 2.0-11.7 % RDA, for W1 and W2, respectively. The
11 lowest predictions were for urban group, while for non-urban one were several times higher. Exceedance
12 the aesthetic limit (risk group 1) included 10 (7) % of the children studied, and contribution of W1 (W2)
13 in this group to RDA was 81%(64 %).
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18 RDA was exceeded for about 2% of children (risk group 2) taking W1, while none for W2. There
19 is certain difference in exceedance values between the study group of children and total population
20 comprising all households with and without children (Table 1), and from which the risk group 1 included
21 less households, i.e. 9 (4) %. However, Zn concentrations for households with children included and
22 whole population were statistically homogeneous among major sites and the difference was just random.
23 This allows for alternative evaluations of parameters with the same significance for which
24 anthropometrical data are not required.
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32 Discussion 33

34 *Zn levels among sites* 35

36 The DW Zn concentrations were low in urban areas if compared to non-urban sites. Within non-urban
37 category peripheral and rural sites were not distinguishable for most parameters of zinc. The above results
38 confirmed substantial differences of DW quality between urban and non-urban sites, similarity of spread,
39 private houses, independent of environment (rural or sub-urban) and contamination at stagnating of
40 similar extent in all site types. The significant differences between urban and non-urban sites were also
41 observed for several metals (Pb, Cd, Al) described earlier (Barton et al. 2002, Barton 2005, Barton
42 2008).
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51 The possible reason of low Zn in U-sites can be that large apartment houses were built in the urban
52 areas few decades ago, at a defined period, and steel-pipelines interior were aged to the same extent. Zinc
53 can be leached off from new zinc plated pipelines in higher amounts than from older ones. During
54 corrosion in water the Zn layer gets thinner or/and is covered with insoluble zinc compounds (carbonates).
55 In both cases, Zn corrosion can be slowed down.
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60 In non-urban sites the mean Zn concentrations were several times higher than in U-sites and
accordingly a substantial number of households exceeded the aesthetic limit for Zn. Private houses in the

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3 non-urban areas were build in different periods and were supplied with the variety of water sources via a
4 variety of pipeline systems available in the market and selected following the local habits, especially end-
5 pipes and taps. Moreover, lifetime of small houses is shorter and they are more frequent rebuild and
6 renewed than large urban apartment houses. Particularly in recent two decades in Poland, there was
7 increasing trend in building new houses and renewing old ones due to economic grow, especially in
8 peripheral and rural areas.
9

10 Besides the possible presence of domain of higher Zn in sites studies, overall Zn distribution
11 profile in DW was not resolved into or not indicated additional modes at higher Zn. However, higher
12 variation of Zn in NU in comparison to U sites is clearly evident from standard deviations and larger
13 positive differences between medians and GMs, and suggests the presence of such a subtle sub-fraction.
14

15 *Water contamination*

16 For most households, a significant, over two-fold increase in Zn concentration was observed during
17 stagnating overnight independently of the site. For a significant part of population, the contamination was
18 not dependent on background Zn reflected by plateau of contamination-concentration relationship. The
19 constant $D_{1,2}$ value at plateau (0.7 mg L^{-1}) presents average characteristic contamination for the sites
20 studied. The presence of plateau means that after overnight stagnation period (a constant parameter
21 designed in this study), the corrosion reached near-by or saturation level in the thinnest interior part of
22 pipeline. Possibly the process was no longer or weakly dependent on water corrosiveness or is slow
23 enough, taking background Zn (in W2) as its rough measure. The contamination with the metal is then
24 mainly related to quality of pipelines near the household tap and less to water properties. Similar plateaus
25 were also observed for contamination with other metals (e.g. Barton 2005 and subsequent parts).
26

27 The relative extent of DW contamination with Zn from internal pipeline/pipe system, expressed by
28 $R_{1,2}$ or $D_{1,2\%}$ values, is comparable to that of cadmium (Barton 2005) or lead. "Zero level" of such a
29 contamination is the ideal expectation for the quality of future pipeline systems. In the case of Zn,
30 however, the contamination did not introduce a significant risk for health, rather on the contrary, may be
31 desired to some extent.
32

33 *Diversity among supply systems*

34 Zn concentrations in household water supplied from five major WTPs in urban Kraków differed
35 significantly in Zn in W1, suggesting that many intermediate factors might be involved. However, this
36 could be expected, since these sources differed in water hardness and mineral composition (MPWiK), and
37 underwent different treatment methods or none. The interior contaminations were also very different. The
38 contaminations were only slightly related to background Zn, but this is just another projection of the same
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3 effects discussed above for the whole population. The highest Zn was in the least treated water from deep
4 well E. The highest background contamination (in W2) was associated with the slowest exchange rate in
5 storage containers. High differentiation in DW Zn between sites is rather typical of zinc in DW, as can be
6 deduced from other studies, e.g. (Xu et al. 2006).
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10 11 *Zinc intake*

12 The Zn intake predictions in this study showed that DW can significantly contribute to Zn intake by 6-
13 year-old children by 9-10% RDA in NU sites but to a negligible extent in U-sites. For 7% of total children
14 or up to 20% from NU sites, where Zn exceeded the aesthetic limit of 3 mg L⁻¹, 1.5 L portion of DW
15 taken in the morning (W1) for whole-day food preparation may introduce 64% of recommended daily Zn.
16 If limit is based on RDA, only 2% of children may be at risk of exceedance of Zn RDA with W1, and
17 none with W2. The estimation is thus similar to that found by Kaluza et al. (2002) for Polish population
18 accounting for 9% contribution of DW to dietary Zn intake by elderly.
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26 In the light of studies suggesting Zn deficiencies worldwide, the Zn in DW contributing even by
27 few percent of RDA is desirable for the populations, and may shift the borderline of deficiency due to
28 dietary food inadequacy. Additional zinc, e.g. from supplementation seems beneficial in diseases such as
29 diarrhoea, chronic hepatitis C, shigellosis, leprosy, tuberculosis, pneumonia, acute lower respiratory
30 infection, and leishmaniasis (Overbeck et al. 2008). It is required especially for the immune system and
31 may give support to control of cold-related diseases typical of children (Fischer Walker et al. 2008). It has
32 been recently found in a children population study that 3 mg daily Zn supplementation reduced the
33 incidence of diarrhoea by 21-42% (p<0.01) (Wuehler et al. 2008). Moreover, Zn seems to have a
34 protective effect against toxicity of several other metals, e.g. copper, lead or cadmium (Rogalska et al.
35 2009), all elements present in drinking water.
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44 Since no particular problems with Zn in DW were encountered, no health-based Zn guideline
45 value was introduced by WHO (2006). However, the lack of obligatory limit may not prevent the
46 excessive Zn in DW used for food preparation (drinks, soups etc.), where unacceptable taste can be
47 masked. It may also be a concern in formula fed infants and people of weakened taste abilities, or others,
48 disabled for self-control.
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53 Apart from prevalence of global Zn deficiencies, there are also studies that can argue for limitation
54 of Zn exposure or overload, however, it may apply rather to supplementation not to the Zn levels observed
55 in drinking water. Zn was placed within nutrients at high risk of excessive intake, for which the margin
56 between the RDA (or actual intake) and UL is very narrow (Grossklaus et al. 2006). It is known that Zn
57 excess can disturb copper intake (Wapnir and Balkman 1991). However, this point can be relevant mostly
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3 to individuals with or at near-deficiency since the population Cu intake was generally adequate. Further,
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5 animal studies have shown that enhanced Zn consumption may cause impairments in cognitive function
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7 (Flinn et al. 2005). The latter finding is questioned, however, and other authors suggested that trace Cu
8
9 but not Zn influenced inflammatory (Becaria et al. 2006) and Alzheimer-like pathology (Sparks et al.
10
11 2006). Due to different metal intakes applied in the above studies these observations may not be in
12
13 opposition.

14
15 Review of the topic literature allows for earning conviction of significance of steady, even low Zn
16
17 intake, which warrants high intake efficiency. Possible disadvantage of Zn intake variation, e.g. adaptive
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19 response, i.e. restriction, was proved by the study of Hunt et al. (2008) in the absence of phytates. It also
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21 appeared, that balance between Zn and other metals (iron, copper, and probably other divalent cations, or
22
23 even selenium) (Mocchegiani et al. 2008) as dietary as well as for the body level seems to be of a key
24
25 importance due to their strong interactions. The effects of large temporary intake variation, e.g. high and
26
27 irregular supplementation, should be studied. This may hypothetically induce adaptive enforcement in
28
29 intake restricting system and resistance, which may not quickly relax and synchronize with further low
30
31 intake level. Although, a true marker Zn deficiency of population is still lacking, and there are many
32
33 attempts (Fischer Walker et al. 2007) and proposals for new markers to be evaluated (Gibson et al. 2008,
34
35 de Benoist et al. 2008). Due to multifactorial linking of Zn with several biochemical processes, strong and
36
37 multiple interactions and adaptations, the future marker should comprise a larger part of Zn-dependent
38
39 equilibrium system rather than its particular member. Current knowledge about these equilibria and
40
41 dynamics of the adaptive mechanisms is yet still limited. Because of increasing usage of Zn and other
42
43 microelemental supplements, nutrient imbalance as well its intake variations should be included as a
44
45 factor of evaluations of nutritional and health status in populations.

46
47 As regards the water, some studies found an association between low groundwater concentration
48
49 of zinc and later development of childhood onset diabetes (Haglund et al. 1996), and on contrary, possible
50
51 protective effect of zinc in DW on 1 type diabetes mellitus (Zhao et al. 2001). On the other hand,
52
53 increased acidity of tap water was associated with many-fold higher risk of type 1 diabetes (Stene et. al.
54
55 2002), but direct factors have not been identified. This possible indirect effect might be associated with
56
57 microorganisms, as the authors suggested. However, increased levels of other heavy metals (not studied
58
59 there) as an effect of enhanced leaching from the ground at increased acidity, corrosion of pipelines, pots
60
61 and bars during food preparation, what was not studied but should also be considered by the authors.

Other comments

Due to lack of safety limit for Zn in DW, the only health-based criteria are dietary recommendations,

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3 RDAs or ULs. An appreciation of DW Zn as a nutrient source is that it is minimal, not much fluctuating
4 pool of food-independent daily portion of Zn intake. Although Zn shows a strong availability synergism
5 with dietary proteins, availability of metals from DW is generally greater than from food, because of lack
6 of many antagonistic interactions observed with the presence of food, on otherwise. Moreover, effectivity
7 of Zn absorption at low intakes is significantly higher than at high Zn intake. This suggests that Zn from
8 DW, albeit non-intentional, can be considered very valuable in a serious population deficit, and whole Zn
9 intake may practically be quantitatively absorbable. In the light of these suggestions, the intake
10 predictions in this paper can be considered as maximal and practically realistic Zn intake from DW in the
11 deficiency, and they can be truer than for other metals for which biological filters are more restrictive.

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20 Unfortunately, pipeline-derived Zn accompanied several toxic metals present as impurities in Zn
21 used for plating or galvanizing. However, it should be emphasised, the end-part of household pipeline
22 system should rather conform quality criteria of metals for kitchen pots. The production of corrosion
23 resistant pipelines and pipes for household water supply seems to be difficult. Thus to this purpose one
24 may propose for the future new water systems the use of pipelines made from or plated with high purity
25 Zn, especially for the thinnest end-part of in-house just before a tap in the kitchen. This additionally can
26 increase their resistance to electrochemical corrosion and lifetime of Zn layer. This could preserve over-
27 night contamination with some lead and cadmium, observed in current systems. And as the best, it can
28 increase Zn intake by population and allow better control extreme cases of Zn deficit and may shift a
29 population borderline of zinc deficiency. Such an intentional use of zinc and iron bars and pots to enforce
30 these nutrients intake was recently studied (Lechtig et al. 2007). This proposal is also in line of very recent
31 attempts to control copper levels in the body - an 'anti-copper campaign' - suspected to be involved
32 (among others) in neuro-degenerative diseases (e.g. Zatta et al. 2009). The proposed system warrants
33 further studies.
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46 47 **Conclusions**

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49 The study evaluated levels of Zn in household drinking water using two different criteria, exceedance of
50 aesthetic limit in DW, and predicted contribution to dietary requirements of 6-year-old children.

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53 Rural and peripheral sites to the urban areas are homogenous for Zn in DW, and in these sites DW
54 contained two-three-fold higher Zn than in urban site. Accordingly, the intake predictions are significant
55 in the nonurban areas amounting to near 10% RDA. The exceedances of aesthetic concentration limit
56 were between 4-7% in total population, and the risk of exceedance RDA was quite negligible.
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60 Contamination with Zn during overnight stagnation was wide-spread throughout all sites and
generally of similar magnitude independently of the background Zn level. Besides attractive nutritional

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3 point that using morning portion of drinking water taken after stagnation can increase Zn intake from DW
4 by 2-3 times, since this was associated with the increase in toxic element concentrations from current
5 pipeline systems, it is not recommended. However, for the future, the use of higher quality zinc for end-
6 part of pipelines was proposed..
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10 In conclusion, over-night contamination of DW from pipelines was common, significant and of
11 similar extent at all sites. DW can be a considered significant contributor to Zn intake by children in non-
12 urban sites and may shift the population borderline of deficiency.
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19 Zachwieja for interest in this study and for comments.
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6 Figure 1. Distribution of Zn concentrations in flashed household drinking water (W2) from urban (U) and
7 non-urban (NU) sites.
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11 Figure 2 Correlation of Zn concentrations in morning (W1) and evening water samples (W2), for
12 urban (U) and non-urban (NU) households.
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16 Figure 3 Mean Zn concentrations in household drinking water according to the supply systems in Kraków.
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18 Figure 4A-B Changes of Zn concentration in DW after overnight stagnation in pipelines as a function of
19 supplied Zn (in W2) for total population. Upper curves - fraction 'a' - contamination, lower curves -
20 fraction 'b' - deposition. Figure A: absolute changes of Zn ($D_{1,2}$) as a difference $W1 - W2$; Figure B:
21 relative Zn changes as a difference in percent ($D_{1,2}\%$) or ratio ($R_{1,2}$) of $W1 : W2$.
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Table II Zinc concentration in drinking water and related parameters †

Group or parameter	Concentration (mg L ⁻¹)								p-level [¶] W1 vs. W2 Concentrations (variances)	Related parameters for major fraction ('a' - contamination, D _{1,2} >0) [‡]					Exceed ance [#] 3 mg L ⁻¹ (per cent)	
	Morning water samples (standing water), W1				Evening water samples (flushed water), W2					Concentration, W2 [‡] (mg L ⁻¹)	Ratio R _{1,2}	Difference D _{1,2} (mg L ⁻¹) D _{1,2} %		W 1	W 2	
	GM	N	SDg	Quartiles	GM	N	SDg	Quartiles				GM	N			GM
	(Median)			(CI)	(Median)			(CI)		(Median)	(N%)	(Median)	(Median)	(Median)		
Urban, U	0.46 (0.53)	359	3.27	0.22 - 1.04 (0.04 - 4.63)	0.14 (0.13)	394	3.79	0.06 - 0.40 (0.01 - 1.95)	*** (*)	0.12 (0.11)	290 (81)	4.73 (3.63)	0.33 (0.37)	66 (72)	3	1
Peripheral, P	0.75 (0.80)	131	4.59	0.30 - 2.28 (0.04 - 14.97)	0.51 (0.51)	139	4.55	0.23 - 1.46 (0.03 - 9.93)	* (0.94)	0.44 (0.48)	89 (68)	2.40 (2.15)	0.40 (0.42)	49 (53)	18	10
Rural, R	0.93 (1.44)	65	5.52	0.49 - 2.83 (0.03 - 26.50)	0.55 (0.85)	71	4.65	0.16 - 1.88 (0.03 - 11.09)	0.06 (0.38)	0.60 (0.87)	44 (68)	2.58 (2.09)	0.62 (0.77)	51 (52)	22	11
Nonurban, NU	0.81 (1.03)	196	4.89	0.36 - 2.48 (0.04 - 18.13)	0.52 (0.61)	210	4.57	0.01 - 4.49 (0.03 - 10.24)	** (0.54)	0.48 (0.54)	133 (68)	2.46 (2.14)	0.46 (0.58)	49 (53)	19	10
Total	0.56 (0.62)	555	3.92	0.26 - 1.38 (0.04 - 8.12)	0.22 (0.24)	604	4.61	0.08 - 0.65 (0.01 - 4.49)	*** (**)	0.18 (0.19)	423 (76)	3.85 (2.75)	0.36 (0.40)	61 (64)	9	4
p [¶] : Uvs.P	** (***)				*** (0.06)					*** (0.61)		*** (***)	0.70 (***)	*** (0.81)		
p [¶] : Uvs.R	*** (***)				*** (0.10)					*** (0.27)		* (*)	** (0.33)	*** (0.52)		
p [¶] : Pvs.R	0.39 (0.27)				0.76 (0.87)					0.20 (0.54)		0.62 (0.67)	0.17 (0.15)	0.69 (0.69)		
p [¶] : Uvs.NU	*** (***)				*** (*)					*** (0.33)		*** (***)	0.11 (***)	*** (0.66)		

†W1, W2 - samples of drinking water collected in the morning and evening, respectively; AM - arithmetic mean; GM - geometric mean; N - number of samples; N% - percent of the major fraction 'a' in whole group, SDg - geometric standard deviation (transformed from SD for Log Zn_concentrations, SDg = 10^{SD Log Zn}); CI - 95% confidence interval.

¶ p-level of t-test for difference in concentrations as logarithms and homogeneity of variances (in parentheses). Notations for p-level, if explicit value was not given: n.s. p≥0.05, * p<0.05, ** p<0.01, *** p<0.001

‡ the concentrations and values of related parameters are given for fraction 'a' (D_{1,2}>0, i.e. W1>W2) – the major fraction of samples. Mean metal concentrations in W1 can be calculated from the equation: W1 = R_{1,2} * W2.

Percent of samples exceeding the limit of 3 mg L⁻¹

Table II Predicted Zn intake via drinking water by 6-year-old children †

Group	N (N%)‡	% RDA **		Intake (mg kg b.wt. ⁻¹ day ⁻¹)		Daily intake (mg day ⁻¹)			
		GM (MED); P90	GM (MED)	GM (MED)	SDg	Quartiles	P10 - P90	P2.5 - P97.5	
W1									
Urban	169	6 (6); 18	0.024 (0.028)	0.56 (0.61)	3.03	0.3 - 1.1	0.1 - 1.83	0.0 - 3.3	
Non-urban	135	13 (16); 81	0.055 (0.066)	1.32 (1.56)	4.64	0.7 - 3.7	0.2 - 8.14	0.0 - 12.5	
Total	304	8 (9); 45	0.035 (0.039)	0.82 (0.88)	3.97	0.4 - 1.8	0.2 - 4.47	0.0 - 9.7	
Risk group 1 ¶	30 (10%)	81 (79)	0.33 (0.29)	8.09 (7.94)	1.46	6.0 - 9.7	5.3 - 12.5	4.9 - 25.9*	
Risk group 2 ¶	5 (2%)	150 (126)	0.63 (0.50)	15.04 (12.64)	1.40	12.5 - 16.5	-	11.4 - 25.9*	
W2									
Urban	170	2 (2); 10	0.008 (0.007)	0.20 (0.18)	3.68	0.1 - 0.5	0.0 - 0.98	0.0 - 3.8	
Non-urban	136	9 (11); 52	0.036 (0.045)	0.86 (1.08)	4.35	0.3 - 2.7	0.2 - 5.19	0.0 - 7.8	
Total	306	4 (4); 34	0.016 (0.017)	0.38 (0.39)	4.75	0.1 - 1.1	0.0 - 3.40	0.0 - 7.2	
Risk group 1 ¶	21 (7%)	64 (63)	0.26 (0.25)	6.38 (6.26)	1.26	5.2 - 7.7	4.8 - 8.9	4.6 - 9.4*	
Risk group 2 ¶	none								

† metal intake was calculated at assumed water consumption 1.5 L day⁻¹ by 6-years-old children and the value of safe level of RDA for Polish population of 4-6 year old children - 10 mg Zn day⁻¹; GM - geometric mean; MED - median; P90 (P10) - 90th (10th) percentile; SDg - geometric standard deviation; P2.5, P97.5 - 95% confidence interval for median was calculated as 2.5 - 97.5 percentiles;

‡ number of children and its percent in group (in parentheses);

* minimum and maximum;

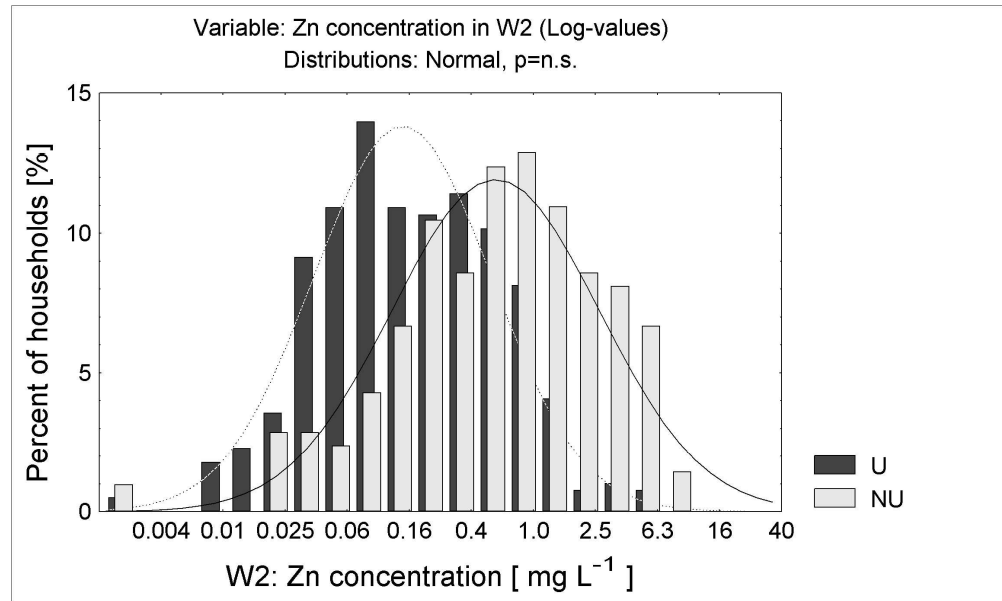
** Note: these values were 10 times daily intakes from next columns

¶ the children from whole population derived via risk criteria: 1) Zn concentration > 3 mg L⁻¹, 2) at Zn intake from DW exceeding the RDA value of 10 mg day⁻¹.

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27 Figure 1. Distribution of Zn concentrations in flashed household drinking water (W2) from urban (U)
28 and non-urban (NU) sites.
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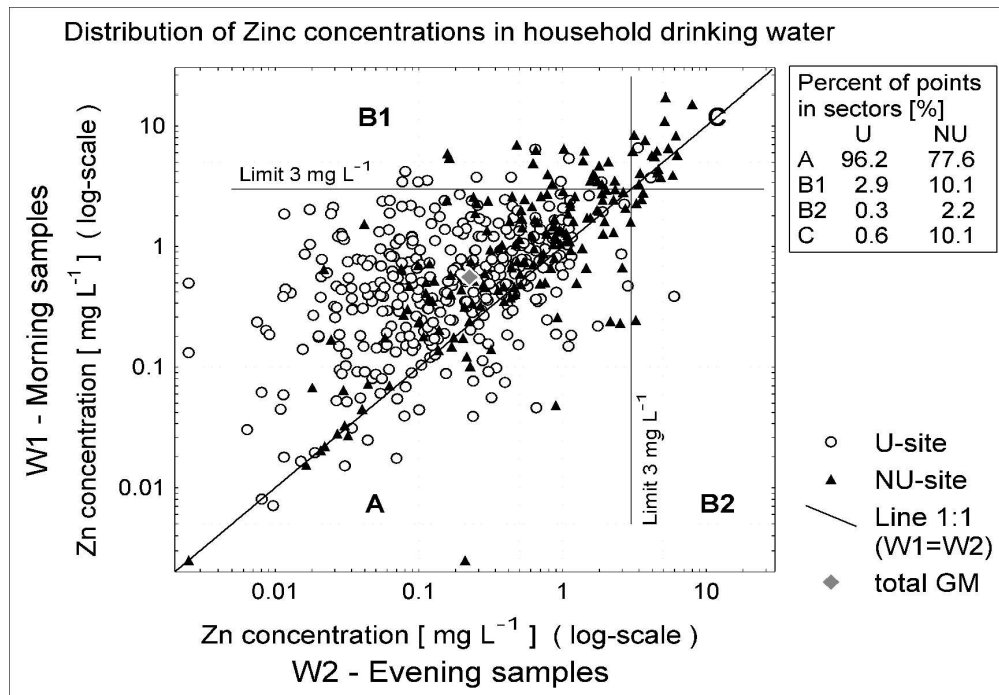


Figure 2 Correlation of Zn concentrations in morning (W1) and evening water samples (W2), for urban (U) and non-urban (NU) households.
1221x842mm (72 x 72 DPI)

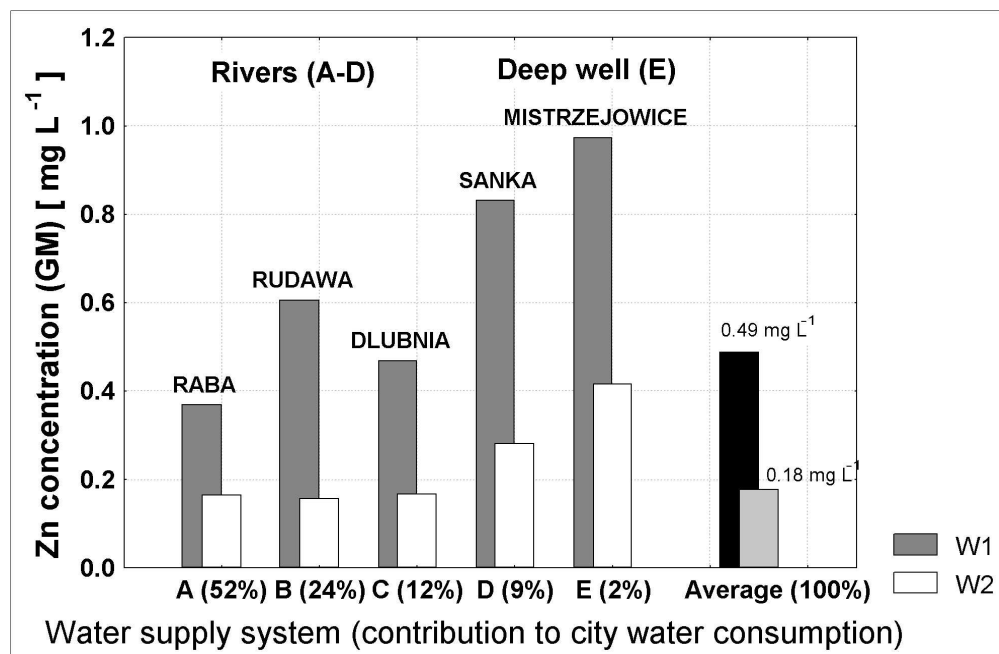
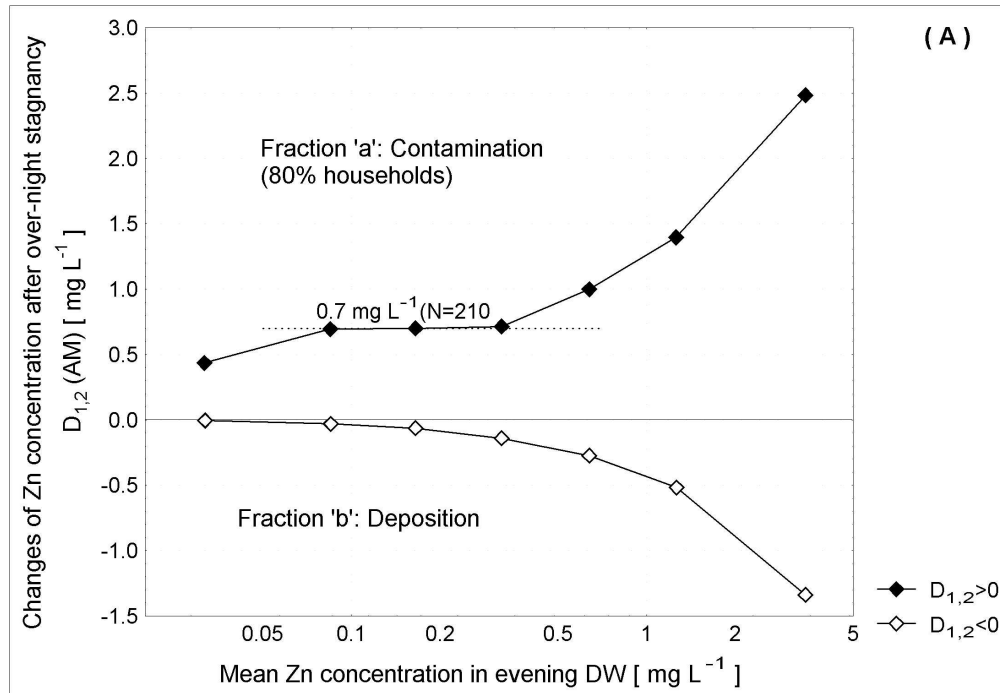


Figure 3 Mean Zn concentrations in household drinking water according to the supply systems in Kraków
699x455mm (72 x 72 DPI)



771x532mm (72 x 72 DPI)

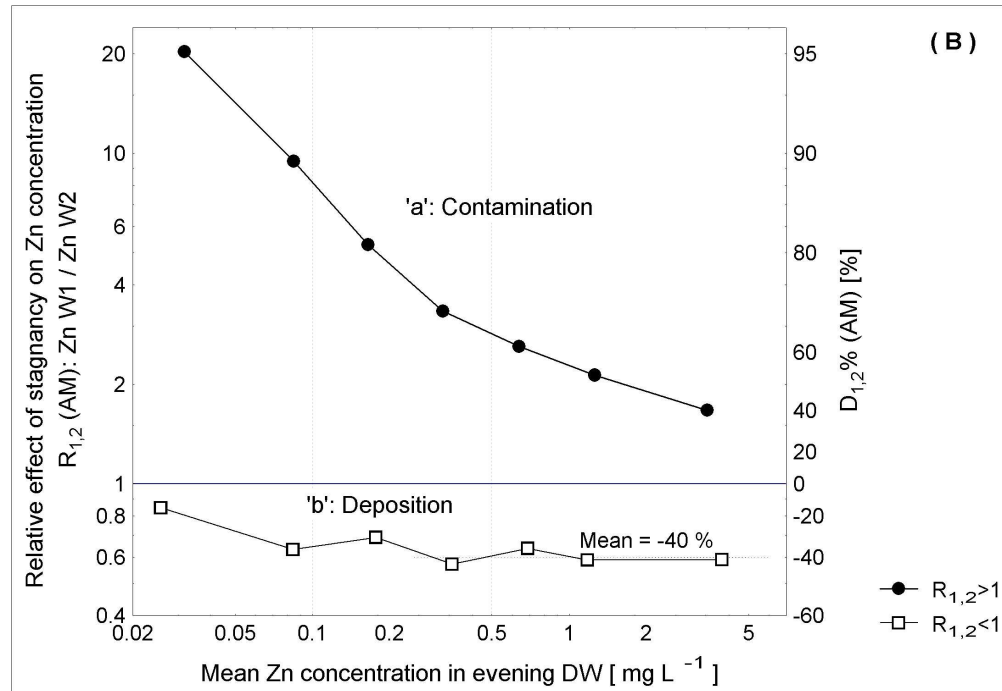


Figure 4A-B Changes of Zn concentration in DW after overnight stagnation in pipelines as a function of supplied Zn (W2) for total population. Upper curves - fraction 'a' - contamination, lower curves - fraction 'b' - deposition. Figure A: absolute changes of Zn (D1,2) as a difference W1 - W2 ; Figure B: relative Zn changes as a difference in percent (D1,2%) or ratio (R1,2) of W1 : W2.
770x530mm (72 x 72 DPI)