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Pavel ČEJKA[1], Tomáš HORÁK[1*], Josef DVOŘÁK[1], Jiří ČULÍK[1], Marie JURKOVÁ[1]
Vladimír KELLNER[1] and Danuša HAŠKOVÁ[1]

MONITORING OF THE DISTRIBUTION OF SOME HEAVY METALS DURING BREWING PROCESS

MONITOROWANIE DYSTRYBUCJI METALI CIĘŻKICH W PROCESIE PRODUKCJI PIWA

Abstract: Herbicides, fungicides and bactericides containing heavy metals used in agriculture make it possible to find these toxic metals in beer. The aim of this work was to monitor the distribution of some toxic heavy metals as Cd, Pb, Ni, Cr, As and Se during beer production. The experiments were observed in pilot plant scale conditions. Except arsenic only a very low fraction of other metals passed into beer. Grain absorbed the majority of the metals (primarily Hg and Pb, the least in the case of As). The yeasts absorbed smaller part of the heavy metals, mainly Ni, As and Se. From the above-mentioned heavy metals only the arsenic was the metal from which the substantial part passed into beer (about two-thirds of added amount). Even after such a large spiking of the brewing water by the concentrations of 0.1÷0.5 mg/dm^3 of individual heavy metals, the content of heavy metals, except for arsenic, in beer remained below the legislative limits.

Keywords: heavy metals, raw materials, brewing process, beer, AAS

The purpose of preparing hopped wort is to transfer extractive substances from the malt and bitter substances from the hops to the solution. An indispensable part of malt extract is formed by minerals, whose total content in the dry matter is about 2÷3%. However, so-called trace elements form just about 0.02% of malt extract. This also applies to toxic metals, which are distributed from the raw materials to the finished beer and brewing residuals. The concentration of metals in the intermediate stages, ie in sweet wort and hopped wort, depends not only on their content in the raw materials (malt, hops and water [1-7]), but also on their ability to transfer into solution during the brewing process [4-11]. This ability is affected by chemical properties of the specific elements and physical-chemical factors, such as temperature, extract concentration, pH and others which act during mashing, wort boiling and during wort cooling. Another decrease occurs during

[1] Research Institute of Brewing and Malting, Brewing Institute Prague, ul. Lípová 15, Praha 2, CZ-120 44, Czech Republic, tel. 224922111
[*]Corresponding author: horak@beerresearch.cz

fermentation. The content of metals in beer in generally very low; thus, in these terms, beer is considered one of the cleanest foods [12-18].

The purpose of this study was to monitor the distribution of selected metals throughout the production process. In the first part of the work, Cu and Al were selected. Increased concentration was studied for these two metals due to their possible presence in some pesticides used to treat hops. The second part simulated the possibility of contamination by toxic metals added to brewing water. It was determined whether this contamination would cause an increased concentration in beer or whether the metal would be separated during the process (eg in spent grain, trub, during fermentation etc.).

Experimental

Technology used

The brews were brewed in a pilot copper four-vessel brewhouse with direct fire gas burner with cast out wort volume of 38 dm^3. The classical method for beer of the Czech type, that is, double mash brewing procedure with a mash-in at 37°C and an ascend with a boiling water addition to 52°C was used in the production. The break at 63°C was 10 min, the saccharification rest for both mashes was 15 min and the mash boil time was 20 min. Distilled water was used as the sparging water. All the brews were prepared as all malt with the wort original extract 10% (°P); they were hopped with hop pellets and were completely matured. Wort boil time was 90 min, the brew was hopped with 12 g of alpha-acids per 100 dm^3 (1 hl) of wort with Saaz hop pellets and hop extract 50 to 50% ratio.

The primary fermentation took place in a stainless fermentation cylinder in a separately cooled refrigerator. Cold wort was fermented at 8°C with first generation of yeast strain W 95 according to VUPS collection. Pitching rate was 0.6 dm^3 of thick yeast slurry per 100 dm^3 (1 hl) of wort. The course of the primary fermentation was controlled so that the temperature would not exceed 11°C. The total duration of the primary fermentation was 8 days, green beer started maturation at the apparent degree of fermentation approx. 71%.

Green beers were matured in modified 30 dm^3 KEG barrels at 1÷2°C for a period of 5 weeks. Finished beer was filtered on pilot plant plate and frame filter and bottled on a pilot plant bottling line under a CO_2 atmosphere with a bottle double pre-evacuation.

Analytical procedures used

Al, Cu, Fe, Mn and Zn were determined by flame atomic absorption spectroscopy (AAS), while Cd, Pb, Ni, Cr, As and Se by flameless AAS (ETA) on Varian 240 FS (2005) instrument [12, 13]. Hg was determined on TMA 254 instrument [8].

Sample take-off points

The samples were taken in the following stages of production: malt, hops, brewing water, mash in, first wort, kettle up, spent grain, cold (hopped) wort, yeast before and after fermentation, green beer, finished beer.

Mass balances of metals

Mass balances of metals were calculated from the following relations:

- malt + water = kettle up + spent grain
- kettle up = first wort + runnings

- wort = kettle up + hops
- cold wort = wort - trub
- cold wort + pitching yeast = green beer + collected yeast

Results and discussion

Study of the distribution of metals after the addition of hops with pesticides containing metals

Economically, the most significant disease of hops is *Pseudoperonospora humuli*. The pathogen attacks the leaves and cones; however, it can also attack the whole plant. It spreads especially during rainy weather, when it can significantly damage or destroy the crops. Copper fungicides, which work on contact and preventively, are still widely used for protection against *Pseudoperonospora humuli*. Another of the significant effective substances used against *Pseudoperonospora humuli* is fosetyl aluminum. It is characterized by a systematic and long-term effect. The metals present in these fungicides pass directly into the brewing process.

Cu and Al were added to the (hopped) wort of the experimental brew, a regular brew was the comparative. The amount to be added was based on specific concentrations of pesticide residue in hops (Tab. 1). The amount to be added was decided to be approximately three times the higher concentration (so that extreme cases would be included) of Cu and Al contents in hops. That concentration is approximately 600 mg Cu/kg and 200 mg Al/kg. A regular dose of hops is approximately 3 g/dm^3 of (hopped) wort. Therefore, the amount added is 5 ppm Cu and 2 ppm Al. Both these metals were added into the (hopped) wort in the form of a sulphate.

Table 1

Pesticides containing heavy metals used for treating hops and allowed in year 2008

Commercial name of treatment	Effective substance	Effective substance content	Concentration applied	Disease
Cuproxat SC	Basic copper sulphate	345 g/dm^3	0.5÷0.75%	*Pseudoperonospora humuli*
Champion 50 WP	Copper hydroxide	77%	0.75%	*Pseudoperonospora humuli*
Ridomil Gold Plus 42,5	Copper oxychloride	400 g/kg	0.35÷0.4%	*Pseudoperonospora humuli*
	Metalaxyl-M	25 g/kg		
Curzate K	Copper oxychloride	77.30%	0.30%	*Pseudoperonospora humuli*
	Cymoxanil	4.00%		
Kuprikol 50	Copper oxychloride	840 g/kg	0.5÷0.75%	*Pseudoperonospora humuli*
Kuprikol 250 SC	Copper oxychloride	420 g/dm^3	0.8÷1.2%	*Pseudoperonospora humuli*
Aliette Bordeaux	Copper oxychloride	250 g/kg	0.4÷0.5%	*Pseudoperonospora humuli*
	Fosetyl-Al	420 g/kg		
Aliette 80 WG	Fosetyl-Al	800 g/kg	0.3% (2.4÷4.5 kg/ha)	*Pseudoperonospora humuli*
Curenox	Copper oxychloride	877 g/kg	0.75%	*Pseudoperonospora humuli*
Funguran-OH 50 WP	Copper hydroxide	77%	0.5÷0.75%	*Pseudoperonospora humuli*
Cuprocaffaro	Copper oxychloride	869.6 g/kg	0.75%	*Pseudoperonospora humuli*
Kocide 2000	Copper hydroxide	53.8%	0.42÷0.56%	*Pseudoperonospora humuli*
Application dose per hectare is 700÷2000 dm^3				

The primary analytical characteristics of malt and hops including Cu and Al contents are listed in Table 2. The metal concentrations measured in the individual stages of the process are presented in Table 3.

Table 2

Selected analytical characteristics and content of Cu and Al in malt and hops

	Malt	**Hops**
Extract [%]	80.6	-
Protein [%]	10.8	-
Bitter substances [%]	-	14.2
Cu [mg/kg]	3.5	275
Al [mg/kg]	16	122

Table 3

Changes in the content of metals during the production process and after the addition of Cu and Al to (hopped) wort [mg/kg], [mg/dm^3]. The values in parentheses are converted to 10% extract

Sample/brew	**Comparative**	**+ 5 ppm Cu**	**Comparative**	**+ 2 ppm Al**
Mash in water	0	0	0	0
Mash in	0.20	0.22	0.82	0.78
First mash	0.06	0.07	0.70	0.66
Second mash	0.06	0.06	0.76	0.78
Mash-out	0.15	0.18	1.22	1.36
First wort	0.40 (0.27)	0.42 (0.28)	1.86 (1.23)	2.02 (1.3)
Last runnings	0.18 (1.14)	0.19 (1.20)	0.40 (3.2)	0.46 (3.7)
Kettle up (10% wort)	0.46 (0.47)	0.48 (0.47)	0.82 (0.80)	0.84 (0.82)
Spent grain	20	22	45	51
Filtered hot (hopped) wort	0.70	2.8	1.6	3.2
Hot (hopped) wort with trub	1.5	5.5	2.9	4.8
Trub	450	1850	680	1320
Cold (hopped) wort	0.10	1.2	0.32	0.62
Finished beer	0.06	0.18	<0.20	0.36

The results show that in the case of both Cu and Al, treating hops with a high dose of pesticides containing these metals will cause a slight, yet significant, increase of their content in beer. However, it is important to note that the dose normally used in production is about a third, so the increase of the concentration of both these metals in beer would be only a few hundredths ppm. The increase of Cu concentration is technologically slightly significant, but insignificant health-wise. That of Al is completely unimportant. A large part of both metals leaves in trub, which can put a strain on the environment.

Monitoring of the distribution of toxic metals during beer production

This part of the study focused on the distribution of metals from barley to malt and the green malt sprouts, the behaviour of heavy metals (Cd, Pb, Ni, Cr, As, Se and Hg) during the course of the brewing process and their distribution between finished beer and brewing residuals.

Heavy metals have the tendency to cumulate in the green malt sprouts (Tab. 4), which can cause problems in its agricultural or industrial use.

Table 4

Concentration of metals in malt sprouts and their ratio to the concentration to malt

Metal	Content [mg/kg]	Ratio of content in malt sprouts to malt
Cu	17÷34	~ 5
Fe	52÷110	~ 3
Zn	72÷99	~ 3
Mn	20÷27	~ 2
Cd	0.04÷0.12	~ 6
Pb	0.2÷0.9	~ 5
Ni	0.6÷1.2	~ 2
Cr	0.9÷1.4	~ 6
As	0.04÷0.10	~ 3
Se	0.06÷0.10	~ 2
Hg	0.008÷0.012	~ 4

The behaviour of toxic metals during production was studied in detail in a regular brew. The metal contents in used malt and hops are listed in first two rows of Table 5. Samples were taken from the brew described upwards and according to the mass balances in chap. "Mass balances of metals", the distribution of the metals from the raw materials (total content 100%) into spent grain, trub, yeast and beer (Fig. 1) was calculated. It was confirmed that toxic metals mostly pass into the residuals (spent grain, trub and yeast), so only a negligible fraction of them pass into beer (except As, of which about half passes into beer). These results essentially correspond with the findings founded in the literature [4-6, 9, 10, 15].

Table 5

Metal content in raw materials, intermediate products and beer after adding toxic metals to brewing water [μg/kg], [μg/dm^3]

Raw materials, intermediate product, beer	Cd	Pb	Ni	Cr	Hg	As	Se
malt (dry matter)	15	41	640	120	2	22	30
hops (dry matter)	30	295	1110	880	11	60	50
water	200	500	300	300	100	300	300
spent grain orig. (dry matter)	40	80	910	278	5	69	78
spent grain (dry matter)	3050	8330	3030	2760	1700	950	3320
% total amount in spent grain	76	95	54	63	96	22	82
yeast orig. (dry matter)	19	55	3500	64	16	390	1400
yeast (dry matter)	680	480	15100	770	850	8170	8400
% total amount in yeast	1	0,2	11	1	2	7	6
finished beer	**> 1**	**9**	**85**	**80**	**> 1**	**210**	**25**

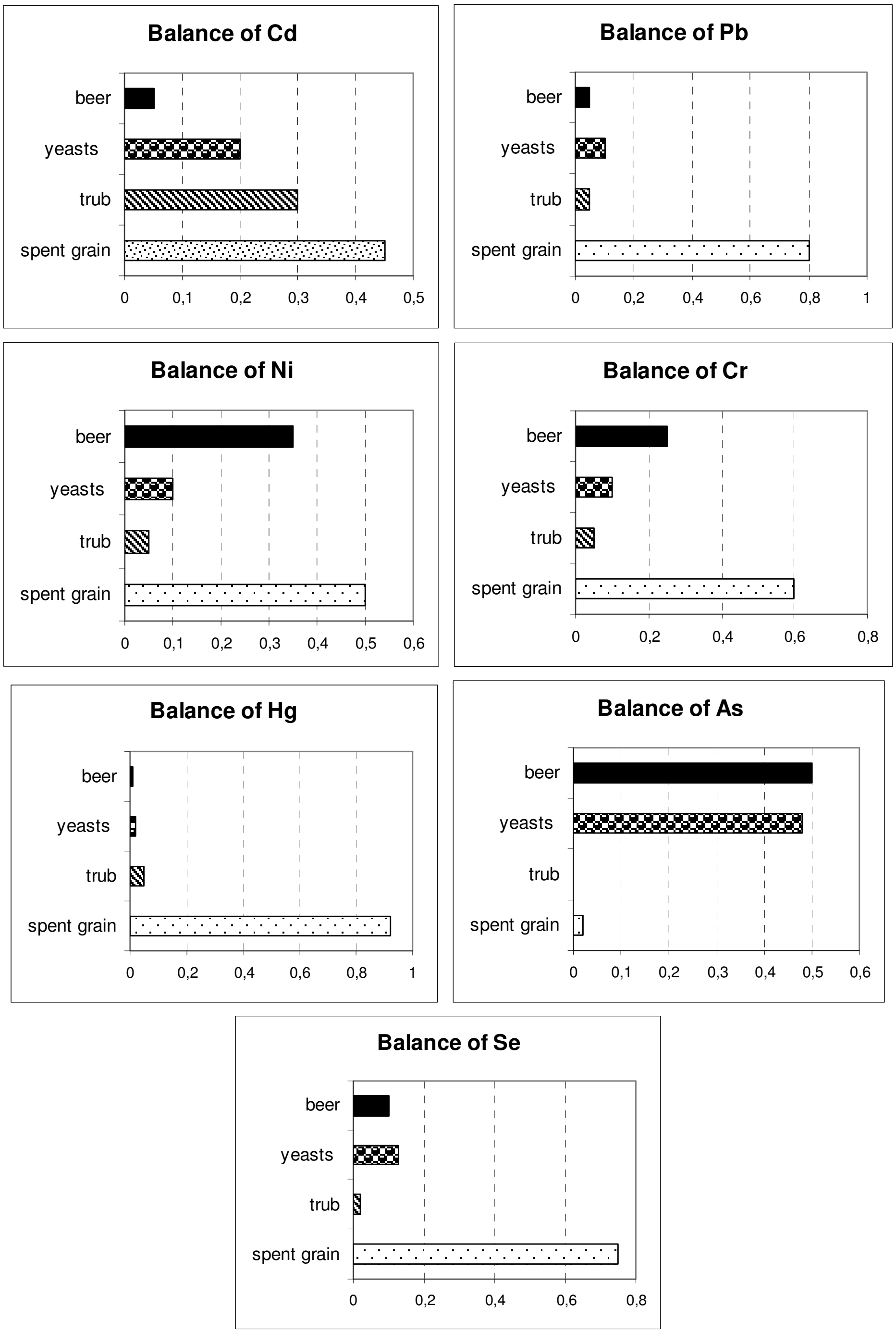

Fig. 1. Distribution of toxic metals from raw materials into brewing wastes and beer, total amount of individual metals in raw materials is 100%

Study of the distribution of toxic metals added to brewing water

A possible contamination by added toxic metals to the process (Tab. 6) was simulated and it was determined whether this contamination would cause an increased concentration in beer or whether the metal would be separated during the process (eg in spent grain, trub, during fermentation etc.).

Table 6

Addition of toxic metals to brewing water

Metal	Addition [μg/dm^3]
Cd	200
Pb	500
Ni	300
Cr	300
Hg	100
As	300
Se	300

The results are shown in Table 5. The amount of metal which passed into beer is graphically represented in Figure 2. As evidenced by results, relatively most of the added amount passes into beer in the case of As (about two thirds) and then for Ni and Cr (about a quarter). Absolute amounts of added metals absorbed by spent grain and yeast were estimated from the mass balances. The results suggest that Hg and Pb have the strongest affinity to spent grain; As the weakest. On the other hand, what cumulates the most in yeast is Ni and then As and Se. An important finding is that even in the case of high contamination of raw materials by toxic metals, their increased content in beer will not be observable (except As) and their level will still be below the allowed limit.

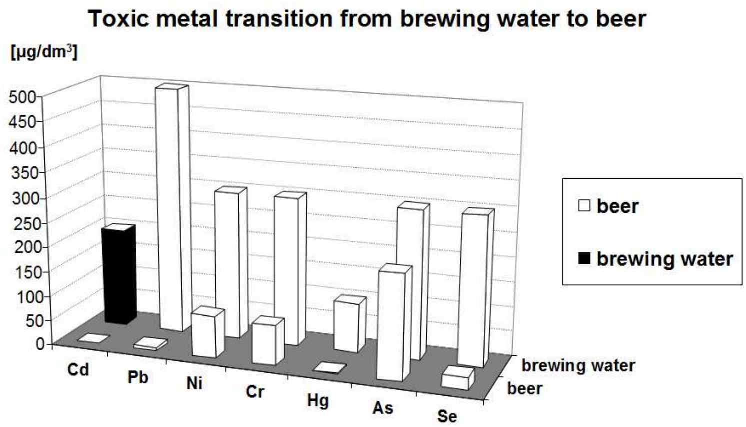

Fig. 2. Amount of metals which passed from brewing water into beer after their addition to brewing water

Conclusions

Based on pilot plant experiments, the monitoring of selected heavy metals during the whole technological process was observed. Treating hops with a high dose of pesticides containing Cu and Al caused a slight increase in their content in beer. However, the concentration increase of both these metals in beer accounts to only a few hundredths or thousandths mg/dm^3. The increase of the Cu concentration is technologically slightly significant, but insignificant health-wise. That of Al is completely unimportant.

Toxic metals, which pass into beer primarily from raw materials, distribute themselves into the waste (spent grain, trub, yeast) during the brewing process most of the time. Therefore, only a negligible fraction of them passes into beer (except As, of which more than a half passes into beer).

A possible increase of a toxic metal at the beginning of the brewing process will affect the beer only in the case of As; other toxic metals are mostly removed with spent grain and also with trub and yeast. Even after a relatively large addition of toxic metals to brewing water, their content in finished beer remained below the allowed limit (except As).

References

[1] Kieniger H. and Boeck D.: Brauwissenschaft, 1979, **32**, 316-320.
[2] Schmidt R., Anderreg P. and Brendl M.: Brauwelt Int., 2004, **24**, 302-305.
[3] Kjer I., Kastel A., Meier-Ploeger A. and Wogtmann H.: Brauwelt, 1994, **14**, 462-468.
[4] Leubolt R., Urban A., Berchtold M., Peter M. and Püspöck J.: Getränke Int., 1991, **45**, 133-139.
[5] Leubolt R., Urban A., Berchtold M., Peter M. and Püspöck J.: Getränke Int., 1992, **46**, 80-83.
[6] Donhauser S. and Jacob F.: Brauwelt Int., 1989, **9**, 259-270.
[7] Donhauser S., Wagner D. and Jakob F.: Monatsschr. Brauwiss., 1987, **40**, 247-256.
[8] Čejka P.: Význam kovových iontů v pivovarsko-sladařském procesu. Kandidátská disertační práce, MBÚ ČSAV, 1987.
[9] Mäder C., Sommer G. and Thurl S.: Monatsschr. Brauwiss., 1997, **50**, 138-141.
[10] Thalacker R., Lich A., Stalz A. and Tascha H.: Brauwelt Int., 1997, **17**, 328-331.
[11] Sharp R. and Williams D.R.: J. Amer. Soc. Brew. Chem., 1995, **53**, 85-92.
[12] Kellner V., Čejka P. and Frantík F.: Kvasny Prum., 1983, **29**, 145-147.
[13] Kellner V., Čejka P. and Frantík F.: Kvasny Prum., 1984, **30**, 121-123.
[14] Dostálek P., Čepička J., Enge J., Koplík R. and Burdová E.: Kvasny Prum., 2001, **47**, 327-330.
[15] Donhauser S., Wagner D. and Jakob F.: Monatsschr. Brauwiss., 1987, **40**, 328-333.
[16] Matusiewicz H. and Mikołajczak M.: J. Anal. At. Spectrom., 2001, **16**, 652-657.
[17] Wyrzykowska B., Szymczyk K., Ichichashi H., Falandysz J., Skwarzec B. and Yamasaki S.: J. Agric. Food Chem., 2001, **49**, 3425-3431.
[18] Runping H., Hongkui Li., Yanhu L., Jinghua Z., Huijun X. and Jie S.: J. Hazard. Mater., 2006, **137**, 1569-1576.

MONITOROWANIE DYSTRYBUCJI METALI CIĘŻKICH W PROCESIE PRODUKCJI PIWA

Abstrakt: Metale ciężkie zawarte w środkach chwastobójczych, grzybobójczych i bakteriobójczych stosowanych w rolnictwie są obecne także w piwie. Celem pracy było oznaczenie stężenia niektórych toksycznych metali ciężkich, jak: Cd, Pb, Ni, Cr, As i Se, powstających podczas produkcji piwa. Badania prowadzono z wykorzystaniem instalacji pilotażowej. Ogólnie stwierdzono małe wartości stężenia metali ciężkich w piwie, wyjątek stanowił arsen. Ziarno akumuluje większość metali (przede wszystkim Hg, Pb i As), natomiast drożdże akumulują mniejsze stężenia metali ciężkich, głównie Ni, As i Se. Z oznaczanych metali ciężkich tylko znaczna część arsenu przeszła do piwa. Stężenie metali ciężkich w piwie, z wyjątkiem arsenu, było mniejsze od dopuszczalnego stężenia wyznaczonego przez normy prawne.

Słowa kluczowe: metale ciężkie, surowce, produkcja piwa, piwo, AAS