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METALS AND POLYCYCLIC AROMATIC HYDROCARBONS (PAHs) IN BEER: A REVIEW

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ABSTRACT

The major cations in beer are potassium, sodium, magnesium, and calcium with the anions, chloride, sulphate, nitrate and phosphate. The minor ones include iron, copper, zinc, manganese, lead, arsenic and phosphorous. These metals are important in beer fermentation because they supply the appropriate environment for yeast growth and influence yeast metabolism. Copper and iron are involved in beer conditioning and ageing through reactions resulting in the formation of reactive oxygen species which readily oxidize organic compounds present in beer, changing the quality of foaming and flavour stability of beer. Small amounts of nitrogen-containing constituents are present and phosphorous is associated with some of these. Only trace amounts of sulphur-containing compounds are present but some of these are very potent flavouring agents. Beer is also subject to PAHs contamination due to the drying and roasting of barley. PAHs have been detected in different types of beverages such as tea, coffee, distilled drinks, wine, and fruit juice. Although PAHs are usually found in low concentrations in foods and beverages, they are one of the main factors that contribute to the onset of cancer in humans. This review covers the determination of levels of metals and polycyclic aromatic hydrocarbons in beer. The review was designed to cover the sources of metals and PAHs in beer as well as functions of metals and methods for the determination of metals and PAHs in beer. It was also designed to give insight to the health concerns of beer consumers.

***Keywords*:** Beer, Metals, PAHs, Atomic spectroscopic techniques, Gas chromatography-mass spectrometry (GC-MS).

INTRODUCTION

Sources of metals and PAHs in beer

Beer is a brewed beverage made principally from malt (germinated barley), hop, water and yeast.
Some of the constituents of beer are derived from the raw materials that survive the brewing process unchanged. Others are the result of the chemical and biochemical transformation of the raw materials during malting, mashing, boiling, fermentation and conditioning. Together, all these constituents make up the character of the beer, but in general, different beer and lagers contain different proportions of the same compounds rather than novel constituents. Nevertheless, accidental or deliberate contamination of beer with micro-organisms other than yeast may well produce new metabolites Beer constituents can be divided into volatile and non-volatile components. The volatile components are responsible for the aroma or bouquet of beer. The non-volatile constituents

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of beer include inorganic salts, sugars, amino acids, nucleotides, polyphenols, and hop resins, together with macromolecules such as polysaccharides, proteins, and nucleic acids [1].

Sources of the metal in beer are classified as endogenous and exogenous. Endogenous sources come from natural components used for beer brewing while exogenous sources are metals that come from substances which are added during brewing and as a result of contamination from brewery equipment used for fermentation, conditioning, filtration carbonation and packing. Therefore, metal content in beer depends on the quality of substrates taken, type of beer brewed and country of origin of the beer [2-6]. Country of origin plays a very crucial role in the metal content of beer as the mineral content of brewing water has long been recognized as making an important contribution to the flavour of beer [7] since more than 90% of beer is water [8]..Tables 1 and 2 show the concentration of major metals, and minor and trace metals respectively in beer of different countries of origin.

Table 1: Concentration of major metals in beer of different countries of origin

	Concentration (mg l)$^{-1}$				
	British	Dutch	German	Spanish	Others
Calcium	40.0 – 140	42.2 – 69.8	3.80 – 108	29.0 – 86.2	16.5 - 111
Potassium	135 – 1100	124 – 648	46.7 – 833	22.9 - 496	17.5 - 442
Magnesium	60.0 – 200	55.5 – 265	23.7 – 266	42.0 - 110	50.0 - 112
Sodium	21.9 – 230	10.4 – 47.6	1.19 – 120	3.95 - 103	4.13 – 66.4

(Source: Pohl, 2008) [9]

Table 2: Concentration of minor and trace metals in beer of different countries of origin

Concentration, mg l–1							
	British	Dutch	German	Norwegian	Polish	Spanish	Others
Aluminium		0.08-0.18	0.05-1.24			0.05-2.22	0.005-6.50
Cadmium			0.0002-0.020			0.031-0.40	0.095-0.68
Barium		0.020-0.032	0.034-0.049			0.014-0.033	0.016-0.068
Copper	0.008-0.80	0.032-0.068	0.019-0.80	0.029-0.050	0.029-0.15	0.024-0.080	0.022-0.16
Iron	0.067-0.50	0.064-0.43	0.040-1,55	0.036.0.93	0.015-0.53	0.096-0.92	0.041-1.06
Manganese	0.033-0.18	0.047-0.30	0.040-0.51	0.046-0.78	0.053-0.47	0.031-0.25	0.032-0.36
Lead			0.003-0.024			0.001-0.006	0.0008-0.003
Strontium		0.23-0.41	0.14-0.30			0.13-0.74	0.13-0.39
Zinc		0.013-0.064	0.013-1.48		0.004-1.2	0.001-0.98	0.007-0.28

(Source: Pohl, 2008) [9].

Beer may also contain contaminants such as polycyclic aromatic hydrocarbons (PAHs) due to the various stages of their production, transportation, packaging, or storage. Environmental pollution and climate change have increased the levels of contaminants in beer including polycyclic aromatic hydrocarbons [10]. These compounds have been found in several environmental matrices such as air, water, soil, sediments and biota [11]. PAHs, which have two or more fused aromatic rings, are formed during incomplete combustion of organic matter [12] and are of special concern due to their carcinogenic and mutagenic potential [13].

Metals and PAHs in beer

In our previous works [14-22], it was evidenced that some Nigerian bitter vegetables such as

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Garcinia kola (bitter cola), *Vernonia amygdalina* (bitter leave), *Azadirachta indica* (neem) and *Gongronema latifolium* (bush buck) could substitute hops in beer brewing. The substitution of hop extracts with *Azadirachta indica* extract was also studied by Eboatu *et al*. (2016) [23] with the conclusion that *Azadirachta indica* extract could be used as a substitute to hop extracts. Consequently, the study on metals and PAHs contents in beers had been extended to beers brewed with these potential hop substitutes. For example, the metal content of these beers was evaluated comparatively with beers brewed with hops by (Okafor, 2016) [15].

The result indicated that the metal content of the beers did not differ significantly. Ubuoh (2013) [24] analyzed metal concentrations in selected canned beers consumed in Owerri Urban, Imo State, Nigeria and found some metals, although, within the maximum allowable daily intake. Moreover, Okafor *et al*. (2020) [19] reported the presence of pyrene (Table 3), one of the 16 USEPA PAHs in a beer brewed with *Garcinia kola* extract in their attempt to investigate levels of PAHs contaminants in beers brewed with Nigerian bitter vegetables as hop substitutes. In Okafor *et al*. (2020) [19], pyrene contamination of beer brewed with *G. kola* was stressed in our review work and the authors warned against the use of *G. kola* as a hop substitute in beer brewing.

Table 3: Concentration of the 16 priority USEPA PAHs in the beer samples

PAH	Concentration (mg/kg)				
	Sample A	Sample B	Sample C	Sample D	Sample E
Naphthalene	Nd	Nd	Nd	Nd	Nd
Acenaphthylene	Nd	Nd	Nd	Nd	Nd
Acenaphthene	Nd	Nd	Nd	Nd	Nd
Fiuorene	Nd	Nd	Nd	Nd	Nd
Phenanthrene	Nd	Nd	Nd	Nd	Nd
Anthracene	Nd	Nd	Nd	Nd	Nd
Fluoranthrene	Nd	Nd	Nd	Nd	Nd
Pyrene	Nd	0.00402	Nd	Nd	Nd
Benzo[a]anthracene	Nd	Nd	Nd	Nd	Nd
Chrysene	Nd	Nd	Nd	Nd	Nd
Benzo[b]fluoranthrene	Nd	Nd	Nd	Nd	Nd
Benzo[k]fluoranthrene	Nd	Nd	Nd	Nd	Nd
Benzo[a]pyrene	Nd	Nd	Nd	Nd	Nd
Dibenzo[a, h]anthracene	Nd	Nd	Nd	Nd	Nd
Indeno[1, 2, 3-c, d]pyrene	Nd	Nd	Nd	Nd	Nd
Benzo[g, h, i]perylene	Nd	Nd	Nd	Nd	Nd

Nd = Not detected, Source (Okafor *et al*, 2020) [19]

Sample A = Beer brewed with isomerized hop extract, Sample B = Beer brewed with *Garcinia kola* extract, Sample C = Beer brewed with *Azadirichta indica* extract, Sample D = Beer brewed with *Vernonia amygdalina* extract, Sample E = Beer brewed with *Gongronema latifolium* extract.

dos Santos *et al*. (2021) [25] in the assessment of PAHs and derivatives in beer using a new cold fiber-solid phase microextraction system in twenty-six beer samples comprising ten dark-roasted, ten Pilsen and six dark-caramel beers found that 9-fluorenone and 9-nitroanthracene were detected in some beers. benzo[b]fluoranthene was the most detected analyte in the beers, and at least one PAH was detected in all the beers analysed by the authours.

FUNCTIONS OF METALS IN BEER

The major metals of beer are Ca, K, Mg and Na. The groups of minor and trace metals are Al, Ba, Cd, Co, Cr, Hg, Mn, Ni, Pb, Sr, P and Zn [9].

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Calcium ion is by far the most influential mineral in the brewing process. Calcium reacts with phosphates forming precipitates leading to the release of hydrogen ions and in turn lowering the pH of the mash. This lowering of the pH is critical because it provides an environment for alpha-amylase, beta-amylase and proteolytic enzymes [26].

Sodium has no chemical effect in beer but it contributes to the perceived flavour of beer by enhancing its sweetness levels from 75ppm to 150ppm, gives round smoothness and accentuates sweetness, which is most important when paired with chloride than when associated with sulphate ions [7]. In the presence of sulphate, sodium creates an unpleasant harshness.

Like sodium, potassium can create a 'salty' flavour effect in beer. It is required for yeast growth and inhibits certain mash enzymes at concentrations above 10mg/L [27].

Magnesium is a very useful metal and an essential mineral to the body that helps to form proteins, produce and transport energy, maintain proper functioning of certain enzymes, and contract and relax muscles. Magnesium ions react similarly to calcium ions, but since magnesium salts are much more soluble, the effect on wort pH is of little consequence. Magnesium carbonate reportedly gives more astringent bitterness than calcium carbonate [28]. Calcium and magnesium chlorides give body, palate fullness, and soft sweet flavour to beer.

Zinc plays an important role in fermentation and has a positive action on protein synthesis and yeast growth. It also impacts flocculation and stabilizes foam, i.e. promotes lacing [26].

Iron helps in the formation of haaamoglobin and myoglobin (oxygen carrying protein), which is found in red blood cells and muscles respectively. Besides this, it is also a part of many proteins in the body. However, iron in large amounts can give a metallic taste to the beer. Iron salts have a negative action at concentrations above 3.2mg/L during wort production, preventing complete saccharification, resulting in turbid worts, and hampering yeast activity [29].

Information on the role of some specific major and minor metals in yeast metabolism and the fermentative and sensory performance of beer is given in Table 4 [2-6, 30, 31].

There are many other metals apart from the aforementioned that could be found in beers in either minor or trace amounts such as Al, Ba, Cd, Mn, Sr, Hg, Ni and As etc. that can perform one function or the other in the brewing process [9].

DETERMINATION OF METALS AND PAHs IN BEER

Methods of determination of metals in beer

It has been reported in several works of literature that methods for the determination of metals in beer are majorly atomic spectrometric techniques. According to some authors, they include flame atomic absorption spectrometry (F-AAS) [32-37], electrothermal atomic absorption spectrometry (ET-AAS) [38-44] Wagner 1995 [40], and inductively coupled plasma optical emission spectrometry (ICP-OES) [34, 43, 45-50] (Matsushige and de Oliveira 1993[45]. Other spectrometric techniques proposed for measuring the total metal contents in beer are integrated atom trap flame atomic absorption spectrometry (IAT-F-AAS) [51], thermospray flame furnace atomic absorption spectrometry (T-FF-AAS) [52] and inductively coupled plasma mass spectrometry (ICP-MS) [53].

The beer sample is first degassed and wet digested before metal determination using any of the aforementioned atomic spectrometric techniques. However, direct inspiration of the beer sample has been achieved in the case of measurements with ICP-OES giving lower signal-to-background ratios and requiring a high supplied power for plasma operation [43, 48-50]. Another approach to beer analysis by ET-AAS without the need for comparatively long sample dissolution or a respective sample dilution has been reported by Vinas *et al*. (2002) [42].

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Table 4: Role of some major and minor metals in brewing and effect on beer flavour.

Metal	Description of its role in brewing and effect on beer flavuor
Calcium	– Mash and wort pH reduction through reactions with phosphates, phytates, peptides, proteins and other mash and wort constituents (important for yeast metabolism and fermentation action, and fermentation enzymes stabilization) –Oxalates precipitation (important for governing yeast cells flocculation) –Content above 100 mg l^{-1} in brewing liquors causes phosphates removal and inadequate yeast growth nutrition supply –Minor effect on beer flavour (recommended concentration in beer: 20–150 mg l^{-1})
Copper	–Oxidation/reduction catalysis action in reactive oxygen-containing species formation (responsible for aerobic beer ageing and flavour stability during storage) –High concentration is toxic and mutagenic to yeasts, causes irreversible beer haze (recommended upper limit in brewing liquors and beer: 0.1 mg l^{-1}).
Iron	–Oxidation/reduction catalysis action in reactive oxygen-containing species formation (responsible for beer quality and flavour stability) –High concentration conveys metallic and harsh beer taste and dark colour due to associations with phenolic substances, results also in yeast activity hampering and haze production (recommended upper limit in wort and beer: 0.1 mg l^{-1})
Potassium	–Charge homeostasis maintenance (required for yeast growth) –Participation in osmoregulation and regulation of divalent cations and phosphates uptake by yeast cells –Concentration above 10 mg l^{-1} has different laxative effects and imparts salty beer taste
Magnesium	–Mash and wort pH reduction (similarly as in case of Ca) –Cell division stimulation and enzymes co-factoring (important for yeast growth and yeast fermentation metabolism) –Protection of yeast cells from disadvantageous effects stemming from ethanol, high temperature and osmotic pressure –High concentration contributes to sour and bitter beer taste (recommended upper limit in beer: 30 mg l^{-1})
Manganese	–Oxidation/reduction catalysis action in reactive oxygen-containing species formation (responsible for beer flavour and colloidal stability) –Proteins solubilization and enzymatic action support (important for proper yeast growth) –High concentration causes unpleasant beer taste (recommended concentration in beer: 0.05–0.2 mg l^{-1})
Sodium	–No specific chemical and metabolic influence –High concentration contributes to sour and salty beer taste; level of 75–150 mg l^{-1} contributes to round smoothness and proper beer sweetness (recommended upper limit in beer: 150 mg l^{-1})
Zinc	–Micronutrient to yeast growth and metabolism, and protein synthesis –Participation in ethanol production as enzymes co-factor –High concentration is damaging to yeasts and affects fermentation; deficiency leads to impaired fermentation progress (recommended concentration in brewing liquors: 0.15–0.5 mg l^{-1})

(Pohl, 2008) [9].

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Determination of PAHs in beer

PAH mix containing naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-c,d]pyrene, dibenzo[a,h]anthracene, and benzo[g,h,i] perylene at 2000.0 mg L^{-1} in methanol:methylene chloride (1:1) is usually used as standard for the determination of PAHs. The analysis method, a chromatographic technique which is usually performed on a chromatograph coupled to a mass spectrometer (GCMS) analyzer in an oven temperature program, starting at 80°C followed by heating at a rate of 30°C min^{-1} to 150°C, an increase to 210° C at a rate of 10°C min^{-1} held for 4 min, an increase to 240°C at a rate of 15°C min^{-1} , an increase to 280°C at a rate of 10°C min^{-1} held for 8 min. Helium (99.999%) carrier gas at a flow rate of 2.5 mL min^{-1} is normally used. The injector is often operated at 270°C in a splitless mode for 3 min. The ion trap is also often operated in the electron impact mode (EI) with 70 eV energy and positive mode. The temperature of the ion source is 250°C and the interface temperature is 300°C while analysis is performed in segment Scan mode (m/z 50-300) depending on the analyte. Temperature regimes, held times and segment scan modes may vary according to make and specification of the analyzer and the condition of the analyte sample,

HEALTH CONCERNS OF METALS AND PAHs IN BEER

The rate of beer consumption increases daily across the world, Nigeria inclusive because of her favourable demographics with a populous and vibrant youth and growing middle class, along with a growing, largely youth population with increased disposable incomes.

Depending on the concentration of metals in beer, various metals may be essential for health with a profound effect on wellbeing. Concerning the nutritional value of metals to humans and according to relatively high contents of some of them in beer, it is accepted that a moderate and reasonable beer consumption can to some extent be a valuable source of recommended daily dietary metal intake [54], although at high concentrations, these metals are usually toxic or exert salt stress on the yeasts as in the case of sodium for example [6].

According to the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA) list of hazardous substances, PAHs ranked 7th in the biennial ranking of chemicals deemed to pose the greatest possible risk to human health [55]. Several PAHs are known to be potential human carcinogens and have been implicated in various cancers [56-58]. They have also been implicated in numerous other toxicological manifestations such as reproductive toxicity, intrauterine growth retardation, learning and intelligent quotient deficit, destruction of oocytes and inflammation of kidney cells [59, 60]. However, the toxicity of metals and PAHs beer arises when they are above the maximum allowable daily intake for humans.

CONCLUSION

Metal ions affect the fermentation, maturation and storage of beer and imply its final flavour and colloidal stability. Considering the nutritious value of metals and the effect they have on beer wholesomeness, information on the total metal content in beer is advantageous to the consumers. Depending on the concentration, various metals may be toxic to the human body or essential for health with a profound effect on wellbeing. Beer contaminated with PAHs poses a great risk to human health as several of them are known human carcinogens and are responsible for various cancers and numerous other toxicological manifestations. Therefore, moderate and reasonable beer consumption can be a valuable source of recommended daily dietary metal intake whereas consumption of beer contaminated with PAHs no matter the concentration, can be toxic to humans.

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