Publication Information:
Journal of Environmental Science and Engineering A (formerly parts of Journal of Environmental Science and Engineering ISSN 1934-8932, USA) is published monthly in hard copy (ISSN 2162-5298) and online (ISSN 2162-5301) by David Publishing Company located at 1840 Industrial Drive, Suite 160, Libertyville, IL 60048, USA.

Aims and Scope:
Journal of Environmental Science and Engineering A, a monthly professional academic journal, covers all sorts of researches on environmental management and assessment, environmental monitoring, atmospheric environment, aquatic environment and municipal solid waste, etc..

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Chinese Database of CEPS, Airiti Inc. & OCLC
Cambridge Science Abstracts (CSA)
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Chinese Scientific Journals Database, VIP Corporation, Chongqing, China
Summon Serials Solutions
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Subscription Information:
Price (per year):
Print $600, Online $480
Print and Online $800

David Publishing Company
1840 Industrial Drive, Suite 160, Libertyville, IL 60048, USA
Tel: 1-323-984-7526, 323-410-1082; Fax: 1-323-984-7374, 323-908-0457
E-mail: order@davidpublishing.com
Digital Cooperative Company: www.bookan.com.cn
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Influence of Air Pollution on the Deterioration of Monuments and Museum Collections

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Abstract: The time has come to recognize the extreme importance of the archaeological sites in Jordan. They provide a wealth of evidence and precise information regarding ancient civilizations and cultures. The safeguarding of this cultural heritage is one of the most urgent priorities, that involve the proper conservation and preservation methods of all artifacts and buildings that have an important artistic and historic value. Conservation is a whole field in regarding the care and treatment of valuable artifacts, both movable and immovable. It has two aspects: (1) the control of the environment—to minimize the decay of artifacts and materials and (2) the treatment of these—in order to stop or slow down any deterioration and to stabilize them where possible against further dilapidation. The museum environment is a limited space dedicated not only to exhibition but also to the appropriate conservation of works of art. This article presents an overview of the types of damage and deterioration that air pollution causes to indoor cultural heritage materials and monuments. It identifies the main damaging air pollutants from outdoor sources to be sulphur dioxide, nitrogen dioxide, ozone and sulphide gases.

Key words: Air pollution, cultural heritage, deterioration, museum.

1. Introduction

For a long time, there has been a large interest in the indoor air quality and its effect on human health and materials. It is well known that high levels of air pollutants such as trace gases and particles can harm human health and have detrimental effects on materials [1-3].

Air pollution as a general term means that there are unexpectedly some particles pollutants with high concentration, which occurs almost everywhere and has long history [4]. Also, it is well known that natural stones are considered as the most durable building materials through ages [5].

Efforts to evaluate indoor conditions have progressed from defining areas of interest to making measurements of potentially harmful agents and more recently, to the modeling of expected pollution levels. Thus, weathering and deterioration of cultural monuments and museum objects became natural process particularly near urban and industrial areas owing to air pollution, which if can’t be stopped, it has to be slow down. Otherwise, humans will not have any cultural heritage to preserve in the future [6-8].

2. Aim of the Study

In this part, the different components and chemical characteristics of ambient air in Amman Citadel Mountain were studied according to national and international standards through realizing several statistical and analytical studies of all industrial activities in Jordan. One can say that the different values of air pollution were increased as a direct result of great development and wide using of energy which lead to presences of negative effects on air quality [9].

3. Justification

Jordan archaeological museum and the castle mountain in general contain important collections that require a controlled indoor environment to ensure...
their preservation and minimize their deterioration. In absence of a controlled indoor environment, these objects would be in great danger. Thus, museums must provide at least the minimum requirements for keeping and preserving objects in their current shapes and conditions. Therefore, it seems important to conduct an inclusive study to cater to the needs of the museum and have it improved and developed in order to portray the best possible image of the Jordanian legacy.

4. Location

The Hill of the Citadel (Jabal al-Qal’a) in the middle of Amman was occupied as early as the Neolithic period, and fortified during the Bronze age (1800 BC). The ruins on the hill today are Roman through early islamic (Fig. 1). The name “Amman” comes from “Rabbath Ammon” or “Great City of the Ammonites” who settled in the region sometime after 1200 BC. The bible records that king David captured the city in the early 10th century BC. Uriah the Hittite, husband of King David’s paramour bathsheba was killed here after the king ordered him to the front line of battle [10].

In ancient times, Amman with its surrounding region was successively ruled by the then-superpowers of the Middle East: Assyria (8th century BC), Babylonia (6th century), the Ptolemy’s, the Seleucids (third century BC), Rome (1st century BC) and the Umayyad (7th century AD). Renamed “Philadelphia” after himself by Ptolemy II Philadelphus, the city was incorporated into Pompey (the province of Syria) and later into the province of Arabia created by Trajan (106 AD). As the southernmost city of the Decapolis, Philadelphia prospered during imperial times due to its advantageous location alongside Trajan’s new trade and administrative road—the Via Nova Trajana (Fig. 2) [11].

When Transjordan passed into Arab rule in the 7th century AD, its Umayyad rulers restored the city’s original name of Amman. Neglected under the Abbasids and abandoned by the Mamlukes, the city’s fortunes did not revive until the late 19th century—under the Ottoman Empire. Amman became the capital of the Emirate of Transjordan in 1921 and the newly-created Hashemite Kingdom of Jordan in 1947. Greater Amman (the core city plus suburbs) today remains by far the most important urban area in Jordan, containing over half of the country’s population or about 3 million out of 5 million people [12].

Fig. 1  Amman citadel.
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5. Materials

The correlation between air pollution and museum objects may not be easy. In preservation, environments are a number of environmental factors, which can cause the degradation of material and artifacts. Among these gaseous pollutants are the most destructive element. Gaseous pollution today is caused primarily by the burning of fuels in power plants, factories, commercial and domestic buildings and automobiles. The two main types of gaseous urban air pollution can be classified either as acidic or oxidizing. Over the years, these two types have merged and now the three main pollutants gaseous found throughout the industrialized world are sulphur dioxide (SO₂), nitrogen dioxide (NO₂) and ozone (O₃) [3].

5.1 Sulphur Dioxide

There is a general consensus that the chemical degradation of natural, calcareous building stone in polluted urban environments is predominantly due to the conversion of calcite to gypsum. The sulphation reaction can be represented in a simplified form [13].

\[
CaCO_3 + SO_2 + 2H_2O + 1/2O_2 \leftrightarrow CaSO_4 \cdot 2H_2O + CO_2
\]  

The initial and end products are well known but little known about the intermediate steps. Fassina [5] has suggested three possible routes in this process depending on different atmospheric conditions. Probably, the most important process is the heterogeneous oxidation of SO₂ in the aqueous phase, which occurs in the atmosphere or on the stone surface. According to others, the prevailing mechanism is related to dissolution of SO₂ in bulk water, present in the stone as condensation in the capillaries or absorbed by hygroscopic salts. Catalysts for this latter reaction can be found in soot and dust in the form of transition metal impurities such as Fe-compounds and Mn-compounds. Dry deposition of SO₂ was found to be the dominant input mechanism in urban areas. The amount of aerosol sulphur which is deposited on vertical sheltered stone surfaces is small in comparison with the contribution of gaseous sulphur. Other studies have shown that there may well be a significant contribution of the deposition of CaSO₄ from the atmosphere to large gypsum accumulations in more or less pure silicate sandstone [5]. These latter observations, together with the manifestation of gypsum layers even on bronze and glass surfaces, confirm that the gypsum constituting the deterioration layer does not always completely originate from the underlying rock [14].

The far more soluble gypsum (2.4 g/L), compared...
to calcite (0.014 g/L), is easily washed away from stone surfaces, which are directly exposed to rainfall. In places sheltered from rain, dirt can accumulate as incrustations strongly bound to the surface with CaCO₃ and CaSO₄. The chemical composition and physical properties of the crust are different from the rest of the stone—higher density, lower permeability to water and total or partial substitution of CaCO₃ by CaSO₄. These differences speed up the decay of the stone, because of [15]:

- Variations in volume: gypsum has a greater volume than the calcite replaces and its generation in cracks and pores at the surface is accompanied by expansive stresses;
- The difference in thermal expansion of the gypsum and the calcite. This difference is further emphasized by the black top layer, caused by fumes and carbonaceous particles, which tend to absorb a larger amount of radiation than white surfaces;
- The reduction of permeability, which will increase water retention and all the corresponding adverse effects.

On masonry materials such as many types of sandstone, granite and brick, dry deposition tends to produce a thin black accumulation. Both laboratory and field studies have shown correlations between the amount of this soiling and the dry deposition of sulphur oxides. It is important to discriminate between the effects of SO₂ and acid rain, since sulphur dioxide comes from local sources while acid rain is the result of long-range transport and chemical transformation of SO₂ from distant sources [3].

5.2 Nitrogen Dioxide

The oxides of nitrogen contribute a significant amount of the total loading of air pollution and of acid rain. However, their reaction products do not seem to be present on carbonate stone to the same degree as those of SO₂. The existence of biological sources of nitrate further complicates the relation between atmospheric NOₓ levels and the occurrence of nitrates on exposed stone surfaces. The difficulty in finding calcium nitrate crystals on exposed stone surfaces is probably due to its very high solubility (2,660 g/L for Ca(NO₃)₂·4H₂O) in water and its hygroscopic nature. NO₂ drastically increases the corrosion rate (indicated by weight gain) of calcareous stones in SO₂-containing atmospheres at high (90%), but not at low (50%) relative humidity. Based on laboratory exposure of calcite powders, it has been proven that NO₂ acts as a catalyst for oxidation of S(IV) to S(VI) at a pure calcite surface, in the presence of molecular oxygen at humid conditions (RH: 90%). Extensive field and laboratory exposure of marble also showed that the corrosion due to nitrates and that caused by sulphates are in the same order of magnitude. Nitrate enrichment on different calcareous stone types (marble, limestone and sandstone) was found to originate mainly from deposition of gaseous HNO₃ and to a lesser extent to dry deposition of NO₂. Because of their high solubility, nitrate salts are transported into the inner part of the stone, where they undergo phase transformations such as crystallization and hydration, depending on the ambient conditions (temperature and relative humidity). This may cause micro cracks in the stone structure and hence, accelerate deterioration [16].

5.3 Ozone

During the past decade, there has been a growing concern about the changes in urban air quality [17]. Ozone is a very reactive gas. Tropospheric ozone causes negative health effects in humans and can deteriorate valuable materials [18-20]. It is 100 times or more fast than the catalytic action of iron and manganese with O₂ [21].

Ozone is produced in the stratosphere at a height of 20-30 km by the action of UV radiation on oxygen. The UV concerned here is of much shorter wavelength than ever penetrates to the surface of the earth [3].

A complementary issue is the effect on building materials particularly in their deterioration and
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...maintenance arising from pollution. Presently, those concerns relate specifically to the rise of pollution from vehicles [17].

Ozone does not play a direct role in the deterioration of limestone, but it is important in its strong oxidizing agent. Ozone has been shown to accelerate the sulphate formation at a calcite surface in an SO$_2$ atmosphere under both humid and dry conditions [21].

Methodology:

The measurement took place in April 2012. It was part of an international pollution monitoring campaign undertaken by the “Accessible Heritage” project at University College London’s Centre for Sustainable Heritage.

As part of the campaign, diffusion tubes to measure sulphur dioxide, nitrogen dioxide and ozone were received. These pollutants are generated outdoor, typically from motor vehicle's fuel combustion or industrial processes.

Two types of tubes were received—one with white and black caps for ozone and the other with white and green caps for sulphur dioxide and nitrogen dioxide simultaneously. Two tubes of each type were provided for reproducibility. The four tubes were exposed on the site (Fig. 3) for four weeks. They were then returned to University College London for analysis.

The tubes (Fig. 4) work by a process called molecular diffusion. The white cap of the tube filters out particles while the colored cap contains the...
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absorbent required for a given compound. During molecular diffusion, compounds will move from an area of high concentration to an area of low concentration. The compounds in the air are at a higher concentration than those in the tube, so, the compounds diffuse into the tube and collect on the absorbent at the end of the tube. Because the compounds are absorbed, the low concentration in the tube is maintained and therefore, diffusion continues.

The rate that the compounds move into the tube is called the uptake rate. This is a known rate and is used in the calculations during analysis. The tubes are clear as light is required during the absorption process. A technique called ion chromatography is used in the lab to determine the concentration of compounds on the tube. This is then used in a calculation with the uptake rate to calculate the average concentration of compounds that were present in the air over the monitoring period.

7. Result

For the Citadel Mountain, the averaged results in ppb (part per billion) are as follows:

1. Ozone (O$_3$): 30.58 ppb;
2. Sulphur dioxide (SO$_2$): 3.08 ppb;

For a reference preservation target of 1 year, the maximal recommended guideline values of these 3 pollutants for indoor collection are as follows:

1. Ozone (O$_3$): 5.0 ppb;
2. Sulfur dioxide (SO$_2$): 3.8 ppb;
3. Nitrogen dioxide (NO$_2$): 5.2 ppb.

Conversion tools can be used to estimate indoor values corresponding to these measurements.

8. Conclusion

This study was undertaken in order to assess air quality at Amman Citadel Mountain which represent the center of the capital of Jordan. Criteria air pollutants that were measured include the main damaging air pollutants from outdoor sources—sulphur dioxide, nitrogen dioxide, ozone and sulphide gases. Basic meteorological parameters were also measured. Findings indicate that most detected air pollutants where below their maximum corresponding national limits set by Jordanian ministry of environment. However, ground ozone was exhibited several incidences where it has exceeded maximum limits set by World Health Organization (WHO). High ozone values go in line with previously published modeling results which predict high values of ground ozone at the capital of Jordan city center.

There is a great concern that ozone may expedite erosion and deterioration rates of rocks at the site. Therefore, a mitigation plan has to be strictly adopted in order to protect the treasures of historic sites in Jordan in general. Findings also indicate that westerly and northwesterly wind dominated wind site during this measurement campaign, which implies that the monitoring site and the city center are impacted by air pollutants that are originated somehow by the traffic jam at the nearby city center.

References


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Per Capita (PC) versus Per Adult Human Unit Method (PAHUM): A Net Assessment of EU28-Population, Family/Household, Food Consumption and Environmental Impact

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Abstract: Communication of Lisbon Strategy sets out an integrated package of measures to deliver more sustainable consumption (including food), better environmental protection, correct population and production evaluations by using appropriate and more meaningful methods. It lays ahead as one of the key challenges for EU28-PC, Adult Equivalent (AE) and conjoint evaluations and implementation are not sufficiently dynamic and forward-looking to drive the performance of methods upwards. Those evaluations do not serve the above purpose. On PC, AE method use overall, voluntary and regulatory instruments are not sufficiently connected and potential synergies among the different instruments are not exploited. Divergent national, international approaches send conflicting signals to producers and consumers. As a result, the full potential of the internal food market of EU28 and its impact on environment are not realized and evaluated on properly identified UNIT basis. Misidentified UNIT for measurement would not give correct results and if one installs his correct assumptions on the wrong unit, the falls results will start following each other. The developed PAHUM-(©1989) and policy approach may integrate the potential of the different policy instruments, helping implement them (gender, age, structure and household size) to food consumption and environmental issues.

Key words: Per capita, per adult human unit, adult equivalents, food, environment.

1. Introduction

The European Union has taken important steps to reach its objectives of growth and jobs. The Lisbon strategy has delivered significant results. Within two years over six million jobs have been created and unemployment has been reduced significantly, especially in Germany. However, the challenge is now to integrate sustainability into this picture. Sustainable development aims at the continuous improvement of the quality of life and well-being for present and future generations in a correct and complete form in its evaluation methodology [1]. This is a key objective of the European Union. Yet, increasingly rapid global changes, from the melting of the icecaps to growing energy and food resource demand, are challenging this objective that need to be revaluated and assessed correctly on unit basis. It is very interesting to indicate that all the evaluations stated and discussed above and all the conclusive predictions are made on PC (unit/criteria) basis. Per capita is not only one of the most used measurement but also one of the misused in every aspect of evaluations of global macro economy including food (organic/conventional) consumption/production predictions. It is virtually in every interest area, including environment (CO2 emission). In people’s daily life, the two words—Per Capita is invoked by academics, business people, news man, TV anchors and politicians. Continued interest in

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using “PC” reflects the pervasive feeling that the unit basis fundamental is happening in the world economy where there are a lot of big issues and evaluations of those interconnected under the umbrella of the term “Per Capita” (Unit). PC evaluations of the individual citizens—the most obvious indicators of measured change are those which impinge most directly on a person’s daily family life activities that is acquiring the necessities of life (food and clothing), making a living and providing for their children to sustain their future and environment. Above challenges and assessments based on PC, Adult Equivalent (AE) and conjoint evaluation are directly linked to people’s way of life that they produce and consume which contribute to global warming, pollution, material use and natural resource depletion including food (organic and conventional). The impacts of consumption (especially food) in the EU are felt globally, as the EU is dependent on the energy imports and natural resources. Furthermore, an increasing proportion of products consumed in Europe are produced in other parts of the world including food. The need to move towards more sustainable patterns of consumption and production is more pressing than ever [2]. Agriculture and fisheries are highly dependent on specific climate conditions. Changes in temperature, amount of CO₂ emission in the atmosphere and the frequency and intensity of extreme weather could have significant impacts on crop yields [3-5].

State of art of the article is implementing developed PAHU method (Copy-right©1989)—(age and gender corrected Per Capita–PC<sub>agc</sub>) to revaluate demographic structure, consumer and food consumption potential of EU28, Candidate States and its safety (and efficacy) as needed for the period of 1999/2010/2020. In addition, practical application and CO₂ emissions are also discussed. It involves systematic attempts to create awareness of error inherent to PC (19.4 percentage unit) food and other goods consumption, consumer evaluations and their impact on society and environment. The other aim is to identify the areas of scientific harmonization of quantitative and qualitative development including family and household evaluations. It may likely to influence the future demographic change of EU, its expansion, environmental policies and strategies. In order to compare the available/calculated data on UNIT basis, researchers have to look into main issues.

1.1 Evaluation of the Food Consumption of Different Households and Impact of Environment

Will the world and EU really need more food? Given the enormously unequal distribution of food today around the planet, one might think that distributing food more equally could solve the food challenge. Yet, even if all the food calories available in the world today were equally distributed across the projected population for the year 2050, no food calories were lost between the farm and the fork. Those calories would still fall short of the UNFAO’s “average daily energy requirements” 2,300 kcal PC/day by more than 200 kcal PC/day. If the current rate of food loss and waste were to remain in 2050, the gap would grow to more than 900 kcal per person (PC/day). In short, current global food availability is insufficient to feed the world in 2050 [6, 7]. How can the world adequately feed more than 9 billion (PC) people by 2050 in a manner that advances economic development and reduce pressure on the environment? This is one of the paramount questions the world faces over the next three decades. Answering it requires a “great balancing act” of two needs, each of which must be simultaneously met.

First, the world needs to close the gap between the food available today and that needed by 2050. UNPD [8] presented the detailed world and EU population prospects and the results were summarized that this gap is in part a function of increasing population and wealth [9]. The United Nations Population Division [8] projected that global population will most likely grow from 7 billion PC in 2012 to 9.3 billion PC by 2050. At least 3 billion PC more people are likely to enter
the global middle class by 2050, and they will almost certainly demand more resource-intensive foods. At the same time, approximately 870 million PC of the world’s poorest people remain undernourished even today. When production falls short of people’s needs, the world’s rich can out-compete the poor and hunger will increase. The second, above evaluations, predictions and figures may depict the reality. However, the correctness of the figures that are evaluated on PC, AE and conjoint evaluations and other methods may need to be confirmed, revaluated and corrected for future strategy and policy determination to eliminate hunger and protect the environment.

1.2 Population Dynamics/Environment Relations

Climate change is one of the greatest challenges humanity face today. Its effects are already felt from strengthened storms and rising sea levels to change temperature and weather patterns. They will only grow worse in the future. Urgent action is needed to reduce emissions to mitigate and adapt to these changes. UN [10-14] is working with governments and other partners to better understanding population dynamics—how they affect the changing climate and how people can become resilient in the face of these changes. Only with this knowledge, policy-makers can take on this gravest challenge. Everyone will be impacted by climate change, especially those who are poor, vulnerable and lacking of resources (food). Consumption drives climate change and different groups of people consume differently. However, many analyses of the impacts of population on climate change fail to take these differences into account. Age structure, household size and spatial distribution all affect PC emissions and also should be integrated into climate change modeling [10-12]. Babies, young children and older people who have past their peak working years consume less and produce fewer greenhouse gas emissions than working-age people. Worldwide, the proportion of older persons is rising, with UNDP projecting an increase in the proportion of people over 60 years of age from 10% in 2005 to 22% in 2050. All things being equal, this will result in a reduction in emissions over time [8]. Developing countries have higher percentage under 19-year old population than developed countries. One cannot assume that 6-month old baby nor < 66-year eat and emit CO₂ as much as 20-65-year old.

To evaluate the ideal of the EU28 and European cities urban areas, it is necessary to interrelate different social perspectives to a widened conception and spatial perspective [15]. The rural/urban distribution of the population is a major determinant of emission levels, though not always in predictable ways [14]. The battle for a sustainable environmental future is being waged primarily in the major cities of the world, where population, economic activity and environmental issues are increasingly concentrated. As cities in the developing world grow, unmanaged urbanization can outpace infrastructure and environmental safeguards, leading to high pollution and CO₂ emissions and increasing vulnerability for residents. Better urban planning is quite essential to poverty reduction. Women’s empowerment and slum improvements could help mitigate greenhouse gas emissions, while also provided resilient and adaptive environments to reduce vulnerability, particularly for impoverished urban dwellers [13, 16].

1.3 Age Structure, Spatial Distribution and Urbanization

Age structure, household size and spatial distribution affect PC emissions and also should be integrated into climate change modeling [10-12]. Babies, young children and older people who have past their peak working years consume less and produce fewer greenhouse gas emissions than working-age people. Worldwide, the proportion of older persons is rising, with UNDP projecting an increase in the proportion of people over 60 years of age from 10% in 2005 to 22% in 2050. All things being equal, this will result in a reduction in emissions over time [8]. Developing countries have higher percentage under 19-year old population than developed countries. One cannot assume that 6-month old baby nor < 66-year eat and emit CO₂ as much as 20-65-year old.

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1.4 Gender and Household Size

Women’s historic disadvantages—their restricted
access to resources and information and their limited power in decision-making make them most vulnerable to the impacts of climate change. Rural women in developing countries are still largely responsible for securing food, water and energy for cooking and heating. Drought, deforestation and erratic rainfall cause women to work harder to secure these resources. They can play an essential role in the climate change negotiation process as well as in the development of sustainable and ecologically sound food consumption, production patterns and approaches to natural resource management [17]. Researchers should not forget that particular groups of people are most vulnerable to impacts of climate change and food consumption: women, children, single, female-headed households and the elderly [11, 14, 17].

In a report published in the journal—“Environment, Development and Sustainability” [18], researchers conclude that the dramatic increase in the number of younger, more affluent people living alone are likely to cause a resource consumption crisis in England and Wales. Their findings should serve as a serious warning to other nations. One-person households increase rapidly: previously, the typical one-person householder was the widow, often on a tight budget and thrifty. The rises in younger, wealthier one-person households have a serious impact on the environment. The number of one-person households in the UK has increased significantly over the last 30 years from 18% of all households in 1971 to 30% in 2001. Experts believe that the figure will increase to 38%, to more than a third of all households by 2026 [19]. This pattern is observed all over EU28. One-person households consume more resources: According to the research, people who live in one-person households are the biggest consumers of energy, land and household goods such as washing machines, refrigerators, televisions and stereos PC. They consume 38% more products, 42% more packaging, 55% more electricity and 61% more gas PC than four-person households. In households of four or more, each person produces 1,000 kilograms of waste annually, while those living alone create 1,600 kilograms of waste each year. One-person households also produce more carbon dioxide PC [19]. It was concluded that because of economies of scale, larger households, while emitting more in total, emit less PC. Therefore, decreases in household size mean more emissions, even without more people [20]. Here, from above stand point, the evaluation of the households on PAHU instead of PC basis may be the best approach when household size, gender and age are considered on defined UNIT basis.

2. Material and Methods

2.1 PC, AE versus PAHU Method Evaluations

PC versus PAHU Method: Redefining PC—PC, AE, conjoint evaluations are currently a somewhat controversial set of units and evaluation methods used by different researchers and scientists in food production consumption, economic and environmental evaluations. One reason for the controversy is that these evaluations cover a wide range of concepts that are often used interchangeably. Failure to recognize and address the problems inherent to error bound PC, “one-size-fits-all accept or reject” approach in food and other goods consumption calculations and projections (which are easy to use) may result in erroneous production and consumption projections, misappropriations of resources and discontent among consumers. It may be extremely important to measure the food consumption of the families/households of developed (EU) and developing (Turkey) countries on a standardized UNIT base that may make them comparable.

2.2 Eliminating Inconsistencies

In general, scientists are looking for a suitable yardstick to measure the level of sustainability of a country. Aim is to evaluate the real consumer potential of a developed or developing state or predicting pollution level by a suitable measuring
instrument which may not be found (PC, Adult Equivalent and Conjoint Measurements etc.). Although the main existing indexes were examined, one had to conclude that none of them seem to fit the needs completely. The main shortcomings are:

1. A limited definition of sustainability;
2. Lack of transparency;
3. High complexity;
4. Absence of regular updates;
5. Inconsistencies of the method and criteria used (Adult Equivalent (AE)).

The developed comprehensive model (PAHU Method/Gender and Age Corrected PCgac) aims to redefine PC for the evaluation of family, household, target groups of developed and developing nations on standardized UNIT basis (20-24-year old man/woman) Furthermore, measure their real food consumption potentials, environmental issues for future planning and eliminate the inconsistencies.

3. Material and Methodology: Concept, Key Innovations

State of the art of this paper is implementing PAHU to evaluate the real consumer potential of EU28 and/or any population or target group (on unit basis). PAHU aims to reduce the errors (19.4 percentage units, Fig. 1) inherent in PC projections for food and other commodities production and consumptions [25-27]. Calculation of PAHU (UNIT = 20-24-year-old) based on Basal Metabolic Body Rates (BMR) to obtain the conversion factors for each age group into PAHU (Table 1) that standardizes any population or a target group on UNIT basis. Since PAHU development and its practical use were presented previously [21-29], the criteria used in the method development are summarized.

![Fig. 1 Per Capita error level—BMB energy requirement differences between PC and PAHU (kcal/d). (Assumed reality—(Series 2) · · · · · · PC vs. Ideal/actual representation—(Series 1) - · · · · · · PAHU) (Rectangle (C) is PC area = 100%; triangle A is < 20 – age group = 7.6% of rectangle; triangle B > 20 – age group = 11.8% of rectangle totalling 19.4% and PAHU is 100 – (7.6 + 11.8) = 80.6% of PC area).](image)
Table 1  Calculated conversion factors of the age groups2.

<table>
<thead>
<tr>
<th>Age groups</th>
<th>Calculated BME3 requirements kcal/day</th>
<th>2 PAHU conversion factors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male female average</td>
<td>Male</td>
</tr>
<tr>
<td>0-4</td>
<td>445.1 432.7 438.9</td>
<td>0.262</td>
</tr>
<tr>
<td>5-9</td>
<td>782.1 780.5 781.4</td>
<td>0.462</td>
</tr>
<tr>
<td>10-14</td>
<td>1,138.6 1,156.1 1,147.4</td>
<td>0.672</td>
</tr>
<tr>
<td>15-19</td>
<td>1,571.5 1,487.9 1,492.5</td>
<td>0.974</td>
</tr>
<tr>
<td>20-24(^1)</td>
<td>1,694.0 1,363.3 1,528.7</td>
<td>1.000</td>
</tr>
<tr>
<td>25-34</td>
<td>1,659.0 1,336.0 1,494.5</td>
<td>0.979</td>
</tr>
<tr>
<td>35-44</td>
<td>1,609.0 1,295.0 1,452.0</td>
<td>0.950</td>
</tr>
<tr>
<td>45-54</td>
<td>1,558.5 1,254.0 1,406.3</td>
<td>0.920</td>
</tr>
<tr>
<td>55-59</td>
<td>1,473.8 1,234.5 1,354.2</td>
<td>0.870</td>
</tr>
<tr>
<td>60-64</td>
<td>1,473.8 1,234.5 1,354.2</td>
<td>0.870</td>
</tr>
<tr>
<td>65-74</td>
<td>1,354.6 1,090.6 1,222.6</td>
<td>0.800</td>
</tr>
<tr>
<td>75+</td>
<td>1,218.0 972.6 1,095.3</td>
<td>0.719</td>
</tr>
</tbody>
</table>

\(^1\) Standard Adult Human Unit (Age 20-24) for male and female BME requirements are 1,694.0 and 1,363.36 kcal/d respectively, averaging 1,528.7 kcal/d. \(^2\) PAHU calculation = Population of the age group × Age group’s conversion factor. Conversion Factor Calculation = Male or Female BME kcal/d: 20-24-year old (PAHU) Male or Female BME kcal/d. \(^3\) Basal Metabolic Energy (BME) is the minimum energy cost of body process, representing the excess of endothermic over exothermic reaction.

3.1 Nutrition and Energy Expenditure for Human Productivity

Method deals with primarily the requirement for a standard reference individual (20-24-year-old = PAHU) BMR energy which are calculated for each “5-year-interval” age-groups. An age group of 20-24 was chosen as a standard adult age group (PAHU or reference person) for both male and female because up to that age, the growth represents the bone and the muscle, whereas, after that, every increase almost always represents fat (Table 1). Economics Nobel Price winner [49] Fogel in 2000 used the terminology “technophysio evolution” in his evaluations and concluded that basal metabolic energy, plus energy used for productivity are essential elements of macro economic production.

3.2 Age and Gender Structure of a Population/Target Group

Selected method design correlates to deviant anthropometry that includes defined age and sex structure along other factors (body weight, height, body frame, environmental temperature etc.) affecting BMR, which are also included in the calculations.

3.3 Selected Anthropometric Criteria

Cut-off points for indicators were selected carefully and all were based on literature and were documented. Comparing to research results can characterize changes and trends on BME within the age/gender groups of the population.

3.4 Calculation Procedure of PAHU

BMR and affecting factors are the criteria used to calculate the PAHU conversion factors for the different age groups (five year-intervals) to standardize a population or a target group under one unit (Table 1) because BMR is an essential part of human vitality. The formula and calculations were based on the long-term research findings [30, 31]. It was suggested [32] a three-fourths power of the body weight is the best correlation between body size and resting metabolism.

It has been considered that \( W^n \) is a measure of physiological body size, or metabolic size, and the values of the exponent, \( n \), should be determined from
the data in question. The relationships may be expressed mathematically as: C = bW^n or log C = log b + log W^n. If C is kcal of basal metabolism and W^n is metabolic size, then the ratio C/W^n should be a statistical constant b determined by Brody in 1945 [30]. Thus, the slope of the curve proved to be 0.73, and the value of b, the ratio C/W^{0.73}, was 70.5 which indicated that, on the average, kcal basal metabolism = 70.5 (W^{0.73}). Brody [30] and Kleiber [31, 32] recommended that the equation be written: kcal of basal metabolism = 70 (W_{kg}^{0.75}) and considered it to be a biological constant applicable not only persons of quite different body builds to all homiotherms (mice and elephant) [33]. The basal metabolism value depends on the biological size and this has been accepted by nutritionists generally.

3.5 Age Groups’ BME Requirements—PAHU versus PC Evaluations and Error Level

PAHU method considers younger, older and gender differences where error bound PC evaluations do not considers those parameters. Its basic objective was to reduce uncertainty and to give definitive stature to the quantities being described. PC is defined—Webster Dictionary: “Equal to each individual, per unit of population and/or for each person”. When data are presented on the basis of PC for production and consumption of commodities including goods and foodstuffs/grain, the assumption is 0-19-year old, (6-month old baby) and 66 to 75+ year-old will produce and consume food/emit CO_2 as much as a mature person (20-65-year old) man and/or woman. The prejudice use of PC hardly been challenged in the literature as it was the only unit that should be used in every aspects of economics, environmental evaluations and food consumption projections. Scientists have to eliminate the error from the beginning of the planning stage. Using the detailed anthropometric criteria is generally neglected in the evaluations. Plotting average BMR kcal requirement values (Table 1) against each age groups, illustrates deleterious assessments are not less than 7.6 percentage units for the age group less than 20 and 11.8 percentage unit for the age group over 25-year respectively (Fig. 1), totalling up to 19.4 in the evaluations. Earlier graphic analysis made by using the calculated findings of (Table 1) by percentage units for each PC as compared to PAHU confirmed the findings [25-29].

4. Results and Discussion

4.1 EU Population Evaluation

Aim involved systematic attempts to create awareness of error inherent to PC (19.4 percentage unit, Fig. 1) food and other goods consumption, consumer evaluations and their impact on society and environment. It includes family and household evaluation likely to influence the future demographic change of EU, its expansion policies and strategies and also to explain the effect of error bound PC evaluations on the EU economic crisis that causes the contractions. To start, each mini market of EU28, candidate country, whole Europe’s PC and PAHU-real consumer potentials are calculated for 1999, 2010 and 2020, summarized and tabulated (Table 2). The expansion of the EU between 1999 and 2010, added 187 million PC and/or 156 million PAHU, (including candidate Turkey). The EU-29 population added up to 561 million PC and 469 million PAHU. In 2020, EU28 plus candidate country (577 million PC and 486 million PAHU), plus other 28 European countries with the dependency of EU-member states, Europe’s consumption potential will go up to 701 million PC and 591 million PAHU. So, Europe (total-56 countries) will be the world’s third largest organized trading, production power and organic/conventional food consumer and polluter after China and India (Table 2). The EU, currently, has to cope with demographic decline, low natural growth and the aging of its population. EU28 policy-makers may have to consider looking into the erroneous use of PC and its effects on the results of the decision-making and policy
Table 2  Europe’s pass and future consumer potential evaluation on Per Capita (PC) and Per Adult Human Unit (PAHU) for the years 1999, 2010 and 2020.

<table>
<thead>
<tr>
<th>Years</th>
<th>Countries (000)</th>
<th>1999</th>
<th>2010</th>
<th>2020</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PC</td>
<td>PAHU</td>
<td>PC</td>
<td>PAHU</td>
</tr>
<tr>
<td>1-EU (15)</td>
<td>374,322</td>
<td>317,342</td>
<td>374,222</td>
<td>317,637</td>
</tr>
<tr>
<td>2-EU (13)</td>
<td>109,996</td>
<td>92,031</td>
<td>110,524</td>
<td>93,069</td>
</tr>
<tr>
<td>3-Candidate (Turkey) (1)</td>
<td>65,599</td>
<td>54,003</td>
<td>76,574</td>
<td>63,583</td>
</tr>
<tr>
<td>4-Dependencies of EU member states. (7)</td>
<td>862</td>
<td>707</td>
<td>985</td>
<td>834</td>
</tr>
<tr>
<td>5-Potential candidate countries (5)</td>
<td>20,310</td>
<td>17,382</td>
<td>21,191</td>
<td>17,999</td>
</tr>
<tr>
<td>6-Future enlargement possibilities (4)</td>
<td>12,395</td>
<td>10,491</td>
<td>12,770</td>
<td>10,683</td>
</tr>
<tr>
<td>7-Micro states (4)</td>
<td>125</td>
<td>107</td>
<td>138</td>
<td>118</td>
</tr>
<tr>
<td>8-Former soviet republics (7)</td>
<td>94,699</td>
<td>80,270</td>
<td>90,676</td>
<td>78,284</td>
</tr>
<tr>
<td>Europe* total (56)</td>
<td>678,278</td>
<td>572,297</td>
<td>675,581</td>
<td>573,207</td>
</tr>
</tbody>
</table>

* Total 56 countries including dependencies.

implications not only in food consumption issues but also in other economic and environmental issues that affect the continuing global and EU economic crisis.

4.2 Grain and Red Meat Consumption Evaluations on Error Based PC

To emphasize the difference between developed and developing countries, two almost equally populated countries are considered: Belgium 10,423,493 and Chad with 10,543,464 populations for the year 2010. Although the population numbers (PC) are similar (0.98% difference), the PAHU population numbers showed huge differences (8,784,050 and 7,840,591 respectively) especially in the age groups, under 20 (22.0% and 57.4% for Belgium and Chad respectively). On the basis of 200 kg/Y world PC grain consumption [26], both Belgium’s and Chad’s total grain requirements would be almost the same, 2,084,698 T/Y and 2,108,692 T/Y respectively. However, on the PAHU basis, the requirements would be 1,756,810 T/Y and 1,568,118 T/Y respectively. Percentage unit deviations of PAHU grain consumption from PC (savings) were 16.6% and 26.9% for Belgium and Chad respectively. Similar saving values for meat were 15.7 and 34.4 percentage unit respectively. Previous evaluations of differences between equally populated Sweden and Zambia for the year 1995 gave similar results and their under age 20 population (24.0% and 61.5% respectively) confirmed the results. These findings illustrate the presence of the big gap between PC and PAHU from the standpoint of projecting organic or conventional grain and also meat consumption of developed and developing countries (Tables 3 and 4).

4.3 Carbon Dioxide Emission of Equally Populated Developed and Developing Countries Evaluation on PC and PAHU

Data reported on Tables 4-7 consider carbon dioxide emissions from the burning of fossil fuels and cement manufacture only but not emission from land use such as deforestation from international shipping or bunker fuels also are not included in national figures (Table 6).

Equally populated Belgium (9.9 T/PC) and Chad (0.04 T/PC) (Table 6) actual CO₂ emissions showed huge differences on both PC and PAHU 1.032, 6, 0.870, 0.042 and 0.032 billion T/Y respectively (Table 4). Differences between PC and PAHU for Belgium and Chad were 0.162 billion tons and 0.010 billion tons respectively. When world CO₂ emissions (4.8 T/PC) (Table 6) are used in evaluations, the differences between Belgium and Chad on PC basis were almost the same (~0.005 billion T/Y) but differences PC and PAHU basis were 0.080 and 0.130
Table 3  Evaluation of the red meat and grain consumption of the almost equally populated countries Chad (developing) and Belgium (developed) on Per Capita and Per Adult Human Unit basis [26].

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Population PC</td>
<td>Belgium</td>
<td>Chad</td>
<td>91,199</td>
<td>0.87</td>
</tr>
<tr>
<td>Population PAHU</td>
<td>8,784,050</td>
<td>7,840,591</td>
<td>943,459</td>
<td>10.74</td>
</tr>
<tr>
<td>Difference, #</td>
<td>1,639,443</td>
<td>2,702,873</td>
<td>1,063,430</td>
<td>8.91</td>
</tr>
<tr>
<td>PAHU, % of the total population</td>
<td>84.27</td>
<td>74.36</td>
<td>9.91</td>
<td></td>
</tr>
<tr>
<td>% deviation of PAHU from total PC pop.</td>
<td>15.73</td>
<td>25.64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percentage of &lt;20-year old in total population</td>
<td>22.00</td>
<td>57.40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PC red meat consumption, ton/year*</td>
<td>309,527</td>
<td>313,140</td>
<td>3,613</td>
<td>1.67</td>
</tr>
<tr>
<td>PAHU red meat consumption, ton/year*</td>
<td>260,886</td>
<td>232,865</td>
<td>28,021</td>
<td>10.70</td>
</tr>
<tr>
<td>Difference between PC and PAHU, ton/year</td>
<td>48,641</td>
<td>80,275</td>
<td>31,634</td>
<td></td>
</tr>
<tr>
<td>PC grain consumption, ton/year*</td>
<td>2,084,698</td>
<td>2,108,692</td>
<td>23,994</td>
<td>1.15</td>
</tr>
<tr>
<td>PAHU grain consumption, ton/year*</td>
<td>1,756,810</td>
<td>1,568,118</td>
<td>188,692</td>
<td>10.7</td>
</tr>
<tr>
<td>Difference between PC and PAHU, ton/year*</td>
<td>327,888</td>
<td>540,574</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* World average PC grain consumption is 200 kg/Y and red meat consumption is 29.7 kg/Y [22].

Table 4  Evaluation CO2 emissions of the almost equally populated countries Belgium (developed), and Chad (developing) on Per Capita and Per Adult Human Unit basis.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Actual CO2 emission, PC T/Y*</td>
<td>9.9</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>CO2 emission PC billion T/Y</td>
<td>1.032</td>
<td>0.042</td>
<td>0.990</td>
</tr>
<tr>
<td>CO2 emission PAHU billion T/Y</td>
<td>0.870</td>
<td>0.032</td>
<td>0.830</td>
</tr>
<tr>
<td>Difference between PC and PAHU T/Y</td>
<td>0.162</td>
<td>0.010</td>
<td></td>
</tr>
<tr>
<td>World CO2 emission, PC/T/Y*</td>
<td>4.8</td>
<td>4.8</td>
<td></td>
</tr>
<tr>
<td>CO2 emission PC billion T/Y</td>
<td>0.501</td>
<td>0.506</td>
<td>-0.005</td>
</tr>
<tr>
<td>CO2 emission PAHU billion T/Y</td>
<td>0.421</td>
<td>0.376</td>
<td>0.045</td>
</tr>
<tr>
<td>Difference between PC and PAHU T/Y</td>
<td>0.080</td>
<td>0.130</td>
<td></td>
</tr>
</tbody>
</table>

* Values from Table 5—(Note: A normal car emits 120-140 g CO2/km).

billion tons for Belgium and Chad respectively due to differences between the PC and PAHU population—1,639,443 and 2,702,873 respectively. Because PAHU calculations considered five year interval age groups and gender differences in its population evaluations on UNIT basis.

4.4 Family/Household Dynamics, Socio-Economic Processes and Their CO2 Emitting Evaluations on PC/AE/PAHU Basis

Families and the households are the main consumer units and demanding source of goods and foodstuffs that need to be evaluated very carefully on unit basis in order to create comparable data. Family and household structures are changing with a steady rise in the number of single-person homes/households and the descending number of family members. This increase is seen in developed-EU and USA, emerging-Turkey and developing economies. One should not forget that household numbers and the number of occupants in the households have great impact on economy, food consumption and carbon dioxide emission. In EU, average household occupant is 2.6. However, this number in Turkey is 4.2. In Eastern Anatolia average household number is 5.2 and in the rural areas, goes up to 7.2 [34]. In order to illustrate the effect of gender and age differences between equally numbered,
two households were picked from the real world of two developing countries. Equal numbered two households with different age and gender structure (Table 5) show that PC and AE calculations can result in unintended deleterious assessments of food (Grain) consumption projections as compared to PAHU.

On the PC basis, the picture looks different (Table 4). Many argue that the household, and not the individual (PC) is the more appropriate unit for measuring emissions. However, above findings indicate that PAHU household emission evaluations (20.9 T/Y and 24.9 T/Y) would be better and more sensitive unit in reflecting the age and gender differences as compared to other (PC—28.8 T/Y and 28.8 T/Y and AE—13.0 T/Y and 15.8 Y/T) units for the Ahmed and Celik families (Table 5) respectively. As indicated, above households generally consume together and often produce/emit together and they are affected by the age and gender composition of the family/household. Since these air pollutants are pertinent to local human health (in particular their high concentrations in urban areas), it cannot withstanding their trans-boundary effects. Reporting their concentrations on PC basis may be informative only. In 2010 [35], on PC basis, the EU-27 average emissions were 7.2 kg PC for ammonia (NH₃), 14.8 kg for NMVOCs, 18.3 kg for nitrous oxides (NOₓ) and 9.1 kg for sulphur oxides (SOₓ) respectively. Since predictions on PC basis is error bound (19.4 percentage unit, Fig. 1), those pollutant values when corrected on PAHU would be 8.6 kg/PAHU, 17.6 kg/PAHU, 21.8 kg/PAHU and 10.9 kg/PAHU respectively.
Table 5 Comparing Household-Aboubakar-Chad and Household-Celik-East Turkey\textsuperscript{1} yearly grain requirements and CO\textsubscript{2} emissions on PC, AE and PAHU basis.

<table>
<thead>
<tr>
<th>Household Aboubakar</th>
<th>Household Celik</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender (age)</strong></td>
<td><strong>PC</strong></td>
</tr>
<tr>
<td>Woman (49)</td>
<td>1</td>
</tr>
<tr>
<td>Boy (15)</td>
<td>1</td>
</tr>
<tr>
<td>Girl (12)</td>
<td>1</td>
</tr>
<tr>
<td>Boy (10)</td>
<td>1</td>
</tr>
<tr>
<td>Girl (7)</td>
<td>1</td>
</tr>
<tr>
<td>Girl (3)</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>6</td>
</tr>
</tbody>
</table>

| **Grain reg. T/Y\textsuperscript{*}** | 1.2 | 0.54 | 0.86 | X | **Grain reg. T/Y\textsuperscript{*}** | 1.2 | 0.66 | 1.04 |
| **CO\textsubscript{2} emissions Ton/Year\textsuperscript{**}** | 28.8 | 13.0 | 20.9 | X | **CO\textsubscript{2} emissions Ton/Year\textsuperscript{**}** | 28.8 | 15.8 | 24.9 |

\textsuperscript{*} World average PC grain consumption [26] is 200 kg and red meat consumption is 29.7 kg;

\textsuperscript{**} CO\textsubscript{2} emissions: world average 4.8 Tons/PC value is used to calculate the household annual CO\textsubscript{2} emission calculations (values are from Table 6).

4.5 Inconsistencies Among and Within Adult Equivocal Evaluations (AE)

One of the most popular method of comparing families’ consumption and other criteria is the adult equivalent (AE) scale that was developed by Friedman as long ago in 1935 [42] which is a generalization of the income PC method [43]. There are different approaches to convert the number of persons in the household to “adult equivalents” by developed concepts and formulas. Different formulae are used in discounting gender and counting children and adults [36, 37 and 44]: (1) Adult Equivalent (AE) was described [44]: first adult in the house = 1; other adults > 13 = 0.5 and child (13 or under) = 0.3; Gender is not considered nor the > 66 age group [36, 37]; PAHU values, from Table 1;

(2) Basciary et al. [45] used adult equivalency scale when creating a poverty map for Azerbaijan with a World Bank project. Adult equivalent children aged below the age of six have been assigned a weight of 0.2, children aged 7-12 have been assigned of a weight 0.3, age 13-17 have been assigned a weight of 0.5 and a weight of 1.0 if the household member is older than 17 years and (3) UN approach was used [46] to treat each child between the ages 0 and 14 as equivalent to half an adult and any person over the age of 14 as 1 adult. In another World Bank, Programmatic Poverty Assessment [47] assumed a scale parameter of 0.8 (individuals of age 18 and below) in 70% of the cost of an adult. None of researchers considered gender nor the > 66 age group.

Above defined AE (AE-1, AE-2 and AE-3), evaluations were compared to PC and PAHUM in Table 6. Results illustrated the inconsistencies exist not only among the AE-1 and AE-2, but AE-3 was also among the PC, PAHU and AE units when the grain requirement and CO\textsubscript{2} emissions for 12-member Egyptian Ahmed Household evaluated and compared. Since AE-2 evaluation age groups were divided into more age groups [45] gave slightly higher values then PAHU but still did not consider gender differences and age > 66 that may be one of the reasons gave higher value than PAHU. Certainly, there are consistency problems not only among EU nations and its institutions but also at the international level that do not use the same definitions. These inconsistencies give too much space for arbitrary decisions that will damage the comparability of the family and household statistical data, along consumer population projections.

Finally recent economic EU crisis need to be fixed
Table 6  List of countries by Per Capita carbon dioxide emissions (Ton/PC/Y) [35, 38-41].

<table>
<thead>
<tr>
<th>Developed countries</th>
<th>1990</th>
<th>2000</th>
<th>2009</th>
</tr>
</thead>
<tbody>
<tr>
<td>Germany</td>
<td>-</td>
<td>10.9</td>
<td>9.1</td>
</tr>
<tr>
<td>Netherlands</td>
<td>11.0</td>
<td>11.2</td>
<td>11.0</td>
</tr>
<tr>
<td>Spain</td>
<td>5.9</td>
<td>7.1</td>
<td>5.8</td>
</tr>
<tr>
<td>Belgium</td>
<td>10.8</td>
<td>11.3</td>
<td>9.9</td>
</tr>
<tr>
<td>EU</td>
<td>8.8</td>
<td>8.2</td>
<td>7.4</td>
</tr>
<tr>
<td>Euro area</td>
<td>-</td>
<td>-</td>
<td>7.4</td>
</tr>
<tr>
<td>World</td>
<td>4.3</td>
<td>4.1</td>
<td>4.8</td>
</tr>
<tr>
<td>Developing countries</td>
<td>1990</td>
<td>2000</td>
<td>2009</td>
</tr>
<tr>
<td>Turkey</td>
<td>2.7</td>
<td>3.2</td>
<td>4.1</td>
</tr>
<tr>
<td>Chad</td>
<td>0.0</td>
<td>0.0</td>
<td>0.04</td>
</tr>
<tr>
<td>Egypt</td>
<td>1.3</td>
<td>2.0</td>
<td>2.6</td>
</tr>
<tr>
<td>High income countries</td>
<td></td>
<td></td>
<td>12.97</td>
</tr>
<tr>
<td>Middle income countries</td>
<td></td>
<td></td>
<td>1.56</td>
</tr>
<tr>
<td>Low income countries</td>
<td></td>
<td></td>
<td>0.214</td>
</tr>
<tr>
<td>Highest emissions countries (year)</td>
<td>1990</td>
<td>2000</td>
<td>2011</td>
</tr>
<tr>
<td>Saudi Arabia</td>
<td>13.2</td>
<td>14.3</td>
<td>19.65</td>
</tr>
<tr>
<td>Australia</td>
<td>17.2</td>
<td>17.2</td>
<td>18.02</td>
</tr>
<tr>
<td>USA</td>
<td>19.1</td>
<td>20.0</td>
<td>17.56</td>
</tr>
<tr>
<td>Russia</td>
<td>13.5</td>
<td>-</td>
<td>12.55</td>
</tr>
</tbody>
</table>

* The data only considers carbon dioxide emissions from the burning of fossil fuels and cement manufacture.

and need a solution, but economists and as it was indicated above politicians can’t decide which way to go. World is rapidly moving toward a period of basic resource scarcity—oil, water, arable land and especially food that will test the states to maintain future good market relations that is compounded by climate shift as it was emphasized. Previously, without common goals and workable multilateral efforts of global institutions, it will not be likely lead to finding a mutual solution of applying error bound PC evaluations that may not make the findings comparable, may not consider population/target groups age and gender structure. The aim is to give the opportunity data to be considered/compared on equalized unit bases that may
Table 7  PC, AE and PAHU evaluation of Egyptian household Ahmed family—(Per Capita = 12)—(with one week food consumption supply), 387.85 Egyptian pound = 68.53 dollars [48]).

<table>
<thead>
<tr>
<th></th>
<th>Egyptian household</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ages</td>
<td>Male or female</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Male</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Female</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>0.3, 0.3, 0.5</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>0.3, 0.3, 0.5</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.3, 0.3, 0.5</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>1.0, 1.0, 1.0</td>
</tr>
<tr>
<td></td>
<td>27</td>
<td>0.5, 1.0, 1.0</td>
</tr>
<tr>
<td></td>
<td>29</td>
<td>0.5, 1.0, 1.0</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>0.5, 1.0, 1.0</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>0.5, 1.0, 1.0</td>
</tr>
<tr>
<td></td>
<td>42</td>
<td>0.5, 1.0, 1.0</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>0.5, 1.0, 1.0</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>% of PC</td>
<td>100.0</td>
</tr>
<tr>
<td></td>
<td>Grain req., T/Y</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>CO2 emissions T/Y*</td>
<td>31.2</td>
</tr>
</tbody>
</table>

* Egyptian family Ahmed’s PC, AEs and PAHU CO2 emissions T/Y calculated from the values given in Table 6; ** AE # (1)—[3, 36, 44] (EUROSTAT, 1999; 2005; 2008) and (OECD, 2012); AE # (2)—[45] Baschieri et al.; AE # 3—[46] Wadan Lal criteria that were used in calculations are described in section 4.5.

eliminate EU Member States “Me first” strategies. PAHU as an alternative method certainly may contribute to the economic and social challenge the people are facing with today. The challenge is achieving a more sustainable society and environmental issues that are evaluated on PC, AE and conjoint assessments.

5. Conclusions

PAHU versus PC method development [50] suggest that there are four building blocks of a theory: constructs, propositions, logic and boundary and conditions/assumptions. Constructs capture the “what” of theories (what concepts are important for explaining a phenomenon), propositions capture the “how” (how are these concepts related to each other), logic represents the “why” (why are these concepts related) and boundary conditions/assumptions examines the “who, when and where” (under what circumstances will these concepts and relationships work). It should also be mentioned here that innovation diffusion as a process of communication where people in a social system learn about a new innovation and its potential benefits through communication channels (such as mass media or prior adopters) and are persuaded to adopt it.

Developed method—PAHU addresses the following problem: How can global and EU social policies be used to enhance social capacities for economic development by evaluating the population not on error bound PC or AE but PAHU/Gender and age corrected PCgac in the process, eroding the intrinsic values of the social ends that policy makers purport to address. The article argues that this requires rethinking social policy away from its conception as a residual category of “safety nets” of development of both developed and developing countries that merely counteract policy failures. Social policy based on population and consumer evaluations should be conceived as involving overall and prior concerns
with social development, and as a key instrument that works in tandem with economic policy including food production and consumption to ensure equitable and socially sustainable development. Major economic growth and improved living standards, rapidly increasing demand for food and other goods that increase the CO₂ emissions are the major issues facing the population. This is compatible with the negative side of production, measured in terms of PC and family evaluations on Adult Equivalent units respectively. The idea to develop a single composite indicator-PAHU/Gender and age corrected PC = PCgac has so far not been taken into work list in scientific community. This deficiency may now be covered. As Albert Einstein ones put it “people cannot solve problems by using the same kind of thinking they used to create them”. Thus, it is time to develop a new society-wide single composite indicator (PAHU) that describes welfare in more sophisticated way than old and primitive PC-GDP and/or PC organic/conventional food consumption/production or PC-CO₂-emission measure. This composite may also guide scientists in next decades towards sustainable world where economy does not exceed the global limits and endanger global ecosystems as today. PAHU = (PCgac) evokes innovation playgrounds not only for researchers but also decision makers of EU. It can well be applied to every EU member country’s/target groups’ food consumption evaluations and environmental issues and problems. In addition, it may have the potential to have an impact on economic evaluations when Genuine Progress Indicator (GPI) and Sustainable Society Indicator (SSI) are used as basis for the societies-replacement of PC-GDP that is needed for the development in economic re-evaluations. The innovative action of PCgac may require shifts in government planning by adding its ecological impacts into the equation.

Acknowledgement

The authors acknowledge the Aydin Doan Foundation for providing financial support and continuous encouragement of scientific activities and education. The authors also wish to acknowledge the editors of environmental for their helpful guiding comments on the manuscript.

References


Per Capita (PC) versus Per Adult Human Unit Method (PAHUM): A Net Assessment of EU28-Population, Family/Household, Food Consumption and Environmental Impact


Opacity Evaluation for Passenger Diesel Vehicle Cars in Tirana

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Abstract: The study includes both the estimation and evaluation of the gas smoke opacity exhausted by a diesel vehicle engine and the opacity control. The evaluation and estimations were accomplished by MAHA software. This research work has been focused in the evaluation of the opacity coefficient and the sources that can influence in opacity value of the vehicles used in Albania.

Key words: Opacity coefficient, pollutant emissions, internal combustion engine, opacimeter.

1. Introduction

Environmental pollution caused from the transport sector is one of the main problems in Albania which rank the last country in Balkan region [1]. Based on it, Albania has a park of 445,952 vehicles of all categories and from this park, 76.6% are passenger vehicle cars. Numbers of vehicles is growing years by years, starting from 1998, numbers of vehicles is changed from 42 vehicles/1,000 inhabitants up to 121 vehicles/1,000 inhabitants as can be seen in the Table 1.

Tirana—as a metropolitan city of Albania, has counted the largest vehicle passenger cars with 35%, where 71% of them were diesel power engine. This park of vehicles is the main source of environmental pollution in Tirana. The engine pollution action manifests prominently in major urban centers which are characterized by a high vehicles density. It is observed that the peak of pollution is in the morning and the evening where the density of cars is too high in the crossroad. The smoke and gas smell in Tirana city is still outstanding issue for compression-ignition engines that keep many unknown data related to noxious origin—PM, CO₂ and VOC, which due to the mixture formation processes complexity as well of combustion process. Based on it, 1,500 persons/year suffer from internal disease related directly to the environmental pollution from transport sector in Albania in accordance to Albanian Health Institute [3].

This can be improved by analyzing the situation of pollution caused from internal diesel engine and reducing the emission scale in accordance to the European standards [4], which can be seen in the Table 2.

Emission standards for passenger cars are summarized in the Table 2. Starting from the Euro 2 stage, EU regulations introduce different emission limits for diesel and petrol vehicles. Diesels engines have more stringent CO standards but allowed higher NOₓ emissions. Petrol-powered vehicles were exempted from particulate matter (PM) standards through the Euro 4 stage, but vehicles with direct injection engines will be subject to a limit of 0.005 g/km for Euro 5 and Euro 6. Traffic-related emissions of fine particles represent one of the main sources of air pollution especially in urban areas. In particular,
Table 2  Emission standards for passenger cars.

<table>
<thead>
<tr>
<th>Tier</th>
<th>Date</th>
<th>CO</th>
<th>THC</th>
<th>NMHC</th>
<th>NOx</th>
<th>HC+NOx</th>
<th>PM</th>
<th>P (#/km)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diesel</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Euro 1†</td>
<td>Jul-92</td>
<td>2.72 (3.16)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.97 (1.13)</td>
<td>0.14 (0.18)</td>
<td>-</td>
</tr>
<tr>
<td>Euro 2</td>
<td>Jan-96</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.7</td>
<td>0.08</td>
<td>-</td>
</tr>
<tr>
<td>Euro 3</td>
<td>Jan-00</td>
<td>0.64</td>
<td>-</td>
<td>-</td>
<td>0.5</td>
<td>0.56</td>
<td>0.05</td>
<td>-</td>
</tr>
<tr>
<td>Euro 4</td>
<td>Jan-05</td>
<td>0.5</td>
<td>-</td>
<td>-</td>
<td>0.25</td>
<td>0.3</td>
<td>0.025</td>
<td>-</td>
</tr>
<tr>
<td>Euro 5a</td>
<td>Sep-09</td>
<td>0.5</td>
<td>-</td>
<td>-</td>
<td>0.18</td>
<td>0.23</td>
<td>0.005</td>
<td>-</td>
</tr>
<tr>
<td>Euro 5b</td>
<td>Sep-11</td>
<td>0.5</td>
<td>-</td>
<td>-</td>
<td>0.18</td>
<td>0.23</td>
<td>0.005</td>
<td>6 × 10¹¹</td>
</tr>
<tr>
<td>Euro 6</td>
<td>Sep-14</td>
<td>0.5</td>
<td>-</td>
<td>-</td>
<td>0.08</td>
<td>0.17</td>
<td>0.005</td>
<td>6 × 10¹¹</td>
</tr>
<tr>
<td>Petrol (Gasoline)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Euro 1†</td>
<td>Jul-92</td>
<td>2.72 (3.16)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.97 (1.13)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Euro 2</td>
<td>Jan-96</td>
<td>2.2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Euro 3</td>
<td>Jan-00</td>
<td>2.3</td>
<td>0.2</td>
<td>-</td>
<td>0.15</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Euro 4</td>
<td>Jan-05</td>
<td>1</td>
<td>0.1</td>
<td>-</td>
<td>0.08</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Euro 5</td>
<td>Sep-09</td>
<td>1</td>
<td>0.1</td>
<td>0.068</td>
<td>0.06</td>
<td>-</td>
<td>0.005**</td>
<td>-</td>
</tr>
<tr>
<td>Euro 6</td>
<td>Sep-14</td>
<td>1</td>
<td>0.1</td>
<td>0.068</td>
<td>0.06</td>
<td>-</td>
<td>0.005**</td>
<td>6 × 10¹¹***</td>
</tr>
</tbody>
</table>

* Before Euro 5, passenger vehicles > 2,500 kg were type approved as light commercial vehicles N1-I;
** Applies only to vehicles with direct injection engines;
*** 6 × 10¹²/km within first three years from Euro 6 effective dates;
† Values in parentheses are conformity of production (COP) limits.

diesel engines were blamed as one of the main contributors for their inherent high particulate emissions. In order to reduce the impact on human health of particulate emissions from vehicles, new stricter emission standards were considered necessary for Europe. Furthermore, research work has been focused at this group of vehicles and evaluation of the opacity factor in Tirana city.

2. Materials and Methods

Evaluation of opacity factor will play an important role to determine the emission scale from transport sector. Opacity is the optical term for the property of stopping light from being transmitted and when it comes to exhaust gases, it corresponds to how opaque they were. The opacity has been measured in percentage where 0% means that all light is transmitted through the media and 100% refers to the case where no light at all can pass it. Opacity is expressed by Eq. (1) which corresponds to the specific effective optical path length OPL as can be seen in Fig. 1:

\[ N = 100 - \tau \] (1)

Where, \( N \) is the opacity in percent and \( \tau \) is the ratio of transmitted light in percentage.

In addition to the evaluation of the opacity, there is a similar optical property named the light absorption coefficient. This property refers to, as the name implies, the ability to absorb light. For a homogeneous matter, the relationship between the light transmission and the light absorption coefficient is described according to the Beer-Lambert law in Eq. (2):

\[ k = \frac{1}{l_A} \cdot \ln \left( \frac{I}{I_0} \right) \] (2)

Where, \( k \) is the light absorption coefficient, \( l_A \) is the effective optical path length and \( \tau \) is the ratio of transmitted light in percent. Fig. 1a shows that OPL is the distance of media that corresponds to the opacity and how the light intensity decreases as the light passes through the sample, evaluated according to the Eq. (3):

\[ N = 1 - \frac{I}{I_0} \] (3)

Where, \( N \) is the opacity of the gas, \( I \) is the measured light intensity and \( I_0 \) is the light intensity if measured without the gas.

Measurement method was realized in accordance to
Fig. 1  (a) The figure shows the basic principle of an opacimeter and (b) the measurement principle of MDO2 opacimeter.

The vehicles registered on or after January 1, 1980, opacity value must meet the requirement values for diesel for passenger cars category. The official emission measurement cycle in diesel vehicle passengers car category consists of at least 3 and a maximum of 5 individual measurements. The last 3 successive measurements are evaluated. After each individual measurement, there is a cleaning time of 5 seconds while the engine rotates in idle. The arithmetic mean value is calculated from the last 3 measurement exhaust strokes:

\[ k_{\text{mean}} = \frac{k_{n=2} + k_{n=3} + k_n}{3} \]  

(4)

Where, \( n \) is the number of the last measurement exhaust stroke.
The measurement has been completed after 3 individual measurements. If each of the individual measurement values of the first 3 measurements has a k-value less or equal with standard, the arithmetic mean k-mean is calculated from the last 3 exhaust strokes. If all 3 k-values from which the mean value was calculated are not smaller than 60% of the k-mean, then the acceleration measurement is completed. Otherwise, the acceleration measurement is continued with two other measurement possibilities.

After the 5th exhaust stroke is done, the acceleration measurement is completed. The arithmetic mean value was calculated from the last 3 exhaust strokes:

\[ k = \begin{cases} < 2.5 \text{ m}^{-1} & \text{for Non Turbo Engines;} \\ < 3.0 \text{ m}^{-1} & \text{for Turbo Engines.} \end{cases} \]

Vehicles registered after January 7, 2008, opacity value must meet the requirement opacity value:
\[ k = < 1.5 \text{ m}^{-1} \]

3. Results and Discussion

The measurement results for evaluation of the opacity are illustrated in the Fig. 4.

The Fig. 4a shows the relation of the opacity coefficient among the production years of the different cars. From the years 1986-1990 and 1991-1995, it is seen that Volkswagen has the largest \( k_{\text{mean}} \) comparing to the Mercedes and other types. From the years 1996-2000 and 2001-2005, it is seen that Mercedes has the largest \( k_{\text{mean}} \) comparing to the Volkswagen and other types. From the years 2006-2010, it is seen that \( k_{\text{mean}} \) is largest for the other cars comparing to Mercedes and Volkswagen.

The Fig. 4b shows the relation between production years and results (pass/fail). The new cars have more opportunity to keep the results “pass” compare to the older cars.

The Fig. 4c shows the influence of the diesel into the opacity coefficient. The results were taken to the Seat Ibiza and the diesel taken at the same company has different scale pollution.

The Fig. 4d shows the types of the car in relation to the pollution scale. From the graph, it can be seen that the Mercedes has the highest \( k_{\text{mean}} \) in comparison to Volkswagen and other cars.

The Fig. 4e shows the relation of the produced
TEST PROCEDURE

Requirements:
- Vehicles registered on or after 01.01.1980
- free accelerations
- gear level neutral
- equipment (MDO) fully connected to the exhaust

START

Rev. to 2,500 rpm +/- 200
and hold for 5 sek

Noisy?

YES

Rev slow to govern speed
hold for max 1 sec

Goverm speed okay??

NO

T=80 grade C

Hold 2,500 rpm

YES

NO

1 Blow Out acceleration

Pause 10 sec

1st acceleration

YES

NO

value=< 60% of limit

NO

2nd acceleration

Pause 10 sec

YES

NO

two good measurements
average=<limit

NO

next acceleration/pause

?? 5 attempts including 1 and

END PASS

END FAIL
Fig. 4  Results of the opacity measurement for different types of vehicles, marks and vehicle age.
year’s car and \( k_{\text{mean}} \) value. The \( k_{\text{mean}} \) value started to be reduced for the new produced year’s car.

This opacity value is a strong function of vehicle age and their type. In relation of the two main factors that influence in the scale of the opacity, the authors have taken into account the quality of the fuels through the opacity value.

4. Conclusions

This research work has investigated that opacity value was extremely high for the vehicles produced before 2002. Vehicles with ages more than fourteen years have very high opacity level and the most problematic groups that cause high pollution level come from the produced years 1980 up to 2002.

It has been seen that Mercedes has the highest opacity level because of their age they were introduced. It is known that the consume of specific engine oil is related with the kilometers done from the engine [7], and this is why pollution in the Mercedes types is in very high levels comparing to the others type of vehicles in Albania.

Another important factor observed from opacity evaluation can come through the quality of the fuels in Albania which is not guarantee as mentioned in the Fig. 4c. Finally, this research work has shown that the opacity value is almost twice time more than the EU standards \( (2.5\text{-}3.0 \text{ m}^{-1}) \) [8].

References


Corncobs as Substrate for Oleaginous
Yeast–Pretreatment via Steam Explosion and Hydrolysis

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Department of Research and Development, University of Applied Sciences Upper Austria, Wels 4600, Austria

Abstract: Corn cobs are a promising lignocellulosic substrate for the production of biofuels like bioethanol via conventional yeast or biodiesel via oleaginous yeast. Pretreatment of the substrate is essential for further hydrolysis and fermentation steps. This study focused on the steam explosion method as pretreatment. Therefore, different steam explosion severities were evaluated. The content of glucan, xylan and Klason lignin was examined. Xylan degraded with increasing severity from 412.7 g·kg⁻¹ (untreated) to a minimum of 127.3 g·kg⁻¹ dry matter (190 °C/30 min). Glucan concentrations increased from 315.1 g·kg⁻¹ (untreated) to a maximum of 371.6 g·kg⁻¹ dry matter (200 °C/20 min). For soluble lignin, an increase could be observed at rising severity, from 145.3 g·kg⁻¹ (untreated) to a maximum of 214.9 g·kg⁻¹ dry matter (190 °C/30 min). Furthermore, the mass recovery was calculated. At harsher pretreatment conditions, a significant mass loss was observed, estimated by the ash content in the recovered dry matter. The lowest recovery rate was observed for SF = 4.13 (190 °C/30 min) with 68.39%. The produced inhibitors were evaluated.

Key words: Corn cobs, steam explosion, lignocellulose, pretreatment.

1. Introduction

The increasing interest in replacing fossil fuels by fuels from renewable sources has led to a considerable amount of research in bio-source technology. Advanced biofuels from sustainable sources, residual and waste materials, fulfil the EU sustainability standards. Lignocellulosic residues (grasses, straw and corn cobs) have great potential as an energy source and raw material supplier. Corn cobs are an agricultural by-product and as a renewable raw material, they pose a potential feedstock for various sustainable industrial biofuel applications (bioethanol, biodiesel and biogas) [1-3]. Beside fertilisation or combustion, corncobs have no specific usage [4]. Corn cobs can be harvested easily and are produced worldwide in large quantities [5, 6]. In 2012, a total of 2,351,370 t of grain maize was harvested in Austria [7].

Corn cobs are a lignocellulosic material composed of cellulose, hemicellulose and lignin. The polymeric fibres of cellulose and hemicellulose consist of monomeric molecules. Cellulose is a linear polymer of glucose subunits. Hemicellulose is a branched heteropolymer built up by different monomeric saccharides, mainly by xylose, arabinose, glucose and mannose. In most plants, the predominant hemicellulose is a polymer of xylose, linked by β-1-4 glycosidic bonds [8]. These polymers are completed by short side chains of organic acids and sugars. Lignin consists of phenolic macromolecules. Cellulose, hemicellulose and lignin are embedded in a complex matrix which is very resistant to enzymatic degradation [1, 9].

Lignocellulosic substrates have to be pretreated to achieve the sugars glucose and xylose. Different pretreatment options are available, for example, the established method of steam explosion (SE) [10]. Steam explosion developed in 1928 by Mason W. H. [11] is known as one of the most efficient pretreatment methods for lignocellulosic materials [12]. The lignocellulosic structure is destroyed by the treatment with high temperature and steam in a reactor followed by a sudden pressure decrease [1].
Hemicellulose can be hydrolysed and degraded by the treatment. The availability of the cellulose for the enzymatic saccharification is enhanced [9]. With a relative distribution of 30%-50% cellulose and 15%-25% hemicellulose, ligneous plants consist of approximately two thirds of potential fermentable sugars [1, 9]. The content of hemicellulose in corn cobs is relatively high. The fibre composition of corn cobs was described in different studies. This substrate consists of 31%-35% cellulose, 30%-35% hemicellulose and 10%-14 % lignin [13-15].

Aim of this study was to determine the relative distribution of cellulose (given as glucan), hemicellulose (described as xylan—the main hemicellulosic polymer) and lignin (as Klason lignin) in corn cobs after SE treatment. Beside the desired improved enzyme accessibility and subsequently the increased sugar yield, this process also causes a significant mass loss. This was observed in numerous studies on various substrates [16-19]. Another effect is the liberation of toxic degradation products during pretreatment and their accumulation in the hydrolysates. Some of these degradation products (acetic acid, formic acid, furfural and 5-hydroxymethylfurfural (HMF) are reported to inhibit further steps of enzymatic hydrolysis and fermentation process [20, 21]. The aim of the pretreatment is to maintain high levels of fermentable sugars (mainly from cellulose), a high degree of degradation of hemicellulose, high mass recovery rates and low levels of potential inhibitory substances. Optimal steam explosion pretreatment conditions for the substrate corn cobs were evaluated.

2. Material and Methods

The corn cobs (zea mays) investigated for this research was obtained from the Austrian Agency for Health and Food Safety (AGES) in Upper Austria (mixture of different varieties, harvested 2012 in Upper Austria). They were partially air dried (dry matter content (DM): 92.8%) at 20 ± 2 °C and chopped up by a garden shredder (Viking GE 260, Kufstein, Austria) to a particle size of 10 mm.

Pretreatment was performed using a laboratory scale steam explosion unit (VAM GmbH Co KG, Linz, Austria), consisting of a high-voltage steam generator, a 15 L decomposition reactor and a collection and steam expansion vessel. Nine hundred grams (DM = 92.8%) of chopped corn cobs and the same mass of water were added to the pressure vessel for each of the conditions. The material was treated at temperatures of 160 °C, 170 °C, 180 °C and 190 °C using 20 min and 30 min residence times and at 200 °C using a 20 min residence time. The SE sample was evacuated immediately after treatment from the collecting vessel and the inner walls were as completely as possible. The wet substrate was weighed and immediately stored in closed plastic boxes and cooled at 4 °C to avoid evaporation.

The intensity of the SE treatment was described as the severity parameter (R₀)—a semiempirical parameter [22]. The severity factor is defined by Eq. (1):

\[
\log(R_0) = \frac{T-100}{14.75} \times t
\]

Where, upon t is the duration of the treatment in min and T the temperature in °C.

Analyses of sugars and acid insoluble lignin (Klason lignin) were conducted before and after SE treatment according to NREL/TP-510-42618 [23]. Saccharides, organic acids and additionally HMF and furfural were quantified using high performance liquid chromatography (HPLC). The HPLC system Agilent Technologies, Santa Clara, 1,200 Series with a Varian Metacarb 87 H column (300 × 7.8 mm) at 65 °C, H₂SO₄ (c = 5 m mol·L⁻¹) eluent and an isocratic flow rate of 0.8 mL·min⁻¹ was used.

DM and ash content were determined by heating the samples at 105 °C and 550 °C overnight. Weight loss of DM and ash compared to untreated substrates was calculated. Crude protein (protein = 6.25 × nitrogen) was determined according to Dumas with a
Corncobs as Substrate for Oleaginous Yeast–Pretreatment via Steam Explosion and Hydrolysis

Dumatherm analyser (Gerhardt GmbH & Co. KG, Konigswinter, Germany). Starch content was determined enzymatically with a test kit (Megazyme International Ireland Ltd., Bray, Ireland) according to the α Amylase/Amyloglucosidase method. Fat was determined gravimetrically according to Bligh and Dyer with chloroform and methanol [24]. The potential inhibitory substances—formic acid, acetic acid, HMF and furfural—for further process steps were determined from the wet SE products at 10% DM in water (1 hour incubation at 75 rpm).

3. Results and Discussion

Chopped corncobs were subjected to 9 different steam explosion conditions. The temperatures ranged from 160 °C to 200 °C with residence times of 20 min and 30 min. The severity of the SE treatment, which combines temperature and time, can be described with the “severity factor”—logR0. For this study, severities from 3.07 (160 °C, 20 min) to 4.25 (200 °C, 20 min) were tested (Table 1). Higher treatment severities lead to visible changes of structure and colour of the biomass—darker products and smaller particle size. These effects are typical for SE treatment [12].

The untreated substrate was composed of 31.52 ± 0.72 % glucan, 41.27 ± 0.72% xylan and 14.53 ± 0.05% Klason lignin in DM. Further compounds were 4.3 ± 1.0 % crude proteins, 2.4 ± 0.2% ash, 0.7 ± 0.1% starch and 0.3 ± 0.0% fat. Table 1 illustrates the relative shares of glucan, xylan and Klason lignin in the dry matter of the untreated and SE pretreated biomass. The lowest level of the Klason lignin was measured for untreated corncobs (14.53%) and the maximum was reached at a severity of 4.13 (21.49%).

In summary, Klason lignin content in DM increased with the severity of the treatment.

Increasing pretreatment severity led to an accumulation of glucan. The maximum content (37.16%) was observed at the highest severity (4.25) evaluated. The increase of the amount of soluble lignin measured for higher severity indicates the formation of pseudo-lignin from xylan degradation at higher temperatures [25-27]. The xylan is degraded at higher severity to 13.72% at 4.13 in comparison to the xylan value—34.59%—of the corn cobs pretreated at soft condition (160 °C, 20 minutes). Xylan showed a significant dependence on the pretreatment severity, which is in accordance with the literature reviewed [26, 28]. The relative shares of the three main components glucan, xylan and Klason lignin changed due to the loss of xylan at harsh conditions. None detailed paper about the effects on the composition of the corn cobs after SE exits but the composition of steam exploded wheat straw showed the same trend [29].

In Table 2, the mass balances of the products obtained after the SE treatment are shown. The mass loss (compared to untreated substrate) was estimated

<table>
<thead>
<tr>
<th>Sample</th>
<th>Severity factor log(R0)</th>
<th>Glucan (%)</th>
<th>Xylan (%)</th>
<th>Klason lignin (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>-</td>
<td>31.51 (0.72)</td>
<td>41.27 (0.72)</td>
<td>14.53 (0.05)</td>
</tr>
<tr>
<td>160 °C/20 min</td>
<td>3.07</td>
<td>32.97 (0.13)</td>
<td>34.59 (0.57)</td>
<td>15.02 (0.16)</td>
</tr>
<tr>
<td>160 °C/30 min</td>
<td>3.24</td>
<td>33.65 (0.43)</td>
<td>31.63 (0.12)</td>
<td>14.57 (0.07)</td>
</tr>
<tr>
<td>170 °C/20 min</td>
<td>3.36</td>
<td>33.26 (0.38)</td>
<td>31.11 (0.87)</td>
<td>15.01 (0.08)</td>
</tr>
<tr>
<td>170 °C/30 min</td>
<td>3.54</td>
<td>32.77 (0.76)</td>
<td>33.22 (0.07)</td>
<td>17.07 (0.08)</td>
</tr>
<tr>
<td>180 °C/20 min</td>
<td>3.66</td>
<td>35.49 (0.96)</td>
<td>23.31 (0.94)</td>
<td>16.28 (0.72)</td>
</tr>
<tr>
<td>180 °C/30 min</td>
<td>3.83</td>
<td>32.01 (0.06)</td>
<td>21.47 (0.44)</td>
<td>19.74 (0.23)</td>
</tr>
<tr>
<td>190 °C/20 min</td>
<td>3.95</td>
<td>36.88 (0.26)</td>
<td>20.83 (0.08)</td>
<td>16.37 (0.58)</td>
</tr>
<tr>
<td>190 °C/30 min</td>
<td>4.13</td>
<td>36.35 (0.00)</td>
<td>12.73 (0.25)</td>
<td>21.49 (0.03)</td>
</tr>
<tr>
<td>200 °C/20 min</td>
<td>4.25</td>
<td>37.16 (0.37)</td>
<td>13.72 (0.96)</td>
<td>17.19 (0.13)</td>
</tr>
</tbody>
</table>
### Table 2: Mass balance of steam explosion pretreatment of corncobs dry matter and mass loss calculation from ash are referred to untreated corncobs (absolute standard deviation in brackets).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Severity factor log(R₀)</th>
<th>DM loss in %</th>
<th>Mass loss calc. from ash %</th>
<th>Ash content in DM in %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.26 (0.06)</td>
</tr>
<tr>
<td>160 °C/20 min</td>
<td>3.07</td>
<td>5.18 (0.18)</td>
<td>-1.13 (1.75)</td>
<td>2.41 (0.04)</td>
</tr>
<tr>
<td>160 °C/30 min</td>
<td>3.24</td>
<td>15.51 (0.13)</td>
<td>5.77 (0.04)</td>
<td>2.52 (0.00)</td>
</tr>
<tr>
<td>170 °C/20 min</td>
<td>3.36</td>
<td>16.00 (0.11)</td>
<td>4.31 (0.05)</td>
<td>2.58 (0.11)</td>
</tr>
<tr>
<td>170 °C/30 min</td>
<td>3.54</td>
<td>18.56 (0.30)</td>
<td>6.98 (1.32)</td>
<td>2.59 (0.04)</td>
</tr>
<tr>
<td>180 °C/20 min</td>
<td>3.66</td>
<td>24.73 (0.05)</td>
<td>11.79 (2.03)</td>
<td>2.65 (0.06)</td>
</tr>
<tr>
<td>180 °C/30 min</td>
<td>3.83</td>
<td>24.84 (0.09)</td>
<td>4.84 (1.81)</td>
<td>2.87 (0.05)</td>
</tr>
<tr>
<td>190 °C/20 min</td>
<td>3.95</td>
<td>26.59 (0.14)</td>
<td>1.49 (0.63)</td>
<td>3.04 (0.02)</td>
</tr>
<tr>
<td>190 °C/30 min</td>
<td>4.13</td>
<td>31.61 (0.07)</td>
<td>8.19 (0.70)</td>
<td>3.04 (0.02)</td>
</tr>
<tr>
<td>200 °C/20 min</td>
<td>4.25</td>
<td>36.34 (0.07)</td>
<td>14.12 (0.04)</td>
<td>3.05 (0.00)</td>
</tr>
</tbody>
</table>

by the ash content in the recovered dry matter. The data obtained shows that mass loss increased with the severity of the SE pretreatment. The ash content conformed to other data [30]. The achieved data (mass loss and increase of the ash content) were consistent with the characteristics from steam exploded wheat straw at different pretreatment conditions [31].

In Fig. 1, the recovered masses of glucan, xylan and lignin in g·kg⁻¹ dry matter input for the different SE conditions are shown (combining mass recovery and relative masses in the DM). Xylan is degraded with increasing pretreatment severity from 412.68 g·kg⁻¹ DM (untreated) to a minimum of 120.73 g·kg⁻¹ DM (SF = 4.13). Fig. 1 indicates that the degradation of xylan increases at temperatures above 170 °C (from 311.63 g·kg⁻¹ DM at SF = 3.36 to 214.69 g·kg⁻¹ DM at SF = 3.83). Higher severities lead to further degradation of xylan. The total mass of glucan recovered after steam explosion treatment decreased from 315.16 g·kg⁻¹ DM (untreated) to a minimum of 236.56 g·kg⁻¹ DM (SF = 4.25). For soluble lignin, a slight increase could be observed at rising treatment severity (145.25 g·kg⁻¹ DM (untreated) to a maximum of 214.91 g·kg⁻¹ DM at SF = 4.13). Similar results were achieved from wheat straw and rice straw after steam explosion [31, 32].

Table 3 illustrates the presence of potentially inhibitory substances for further process steps (formic acid, acetic acid, HMF and furfural) in the hydrolysates of the SE products (10% DM in water, 1 hour incubation at 75 rpm). The quantities of the inhibitors increased with the severity of the SE treatment and reached their highest levels (formic acid, HMF and furfural) at SF = 4.13. The maximum value for acetic acid was observed at SF = 4.25. Also the inhibitor quantities from other lignocellulose (rice straw, sunflower stalks and poplar wood) increased at harsher steam explosion condition, altogether the inhibitor quantities were lower—a phenomenon depends on the composition of the lignocellulose itself [28, 32, 33].

The increased levels of these substances can be explained by the formation of degradation products during thermal treatment of lignocellulosic plants [34-36]. Low amounts of inhibitors are favourable for hydrolysis and fermentation [20, 21]. At high inhibitor concentration, detoxification measures (drying) must be applied [37].

### 4. Conclusions

This study has shown that corncobs are a potential feedstock for uncatalysed steam explosion pretreatment and in consequence, a substrate for biofuel production by fermentation (bioethanol via conventional yeast or biodiesel via oleaginous yeast). A harsh pretreatment led to an extensive decomposition of the lignocellulosic fibre matrix and reduced contents of xylan. This ensures an excellent enzyme-to-substrate accessibility...
Corncobs as Substrate for Oleaginous Yeast—Pretreatment via Steam Explosion and Hydrolysis

Fig. 1  The recovered masses of glucan, xylan and lignin (in g·kg⁻¹ dry matter input).

Table 3  Contents of potential inhibitory substances at different pretreatment conditions.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Formic acid in g/L</th>
<th>Acetic acid in g/L</th>
<th>HMF in g/L</th>
<th>Furfural in g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>0.00</td>
<td>0.32</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>160 °C/20 min</td>
<td>0.11</td>
<td>1.39</td>
<td>0.09</td>
<td>0.00</td>
</tr>
<tr>
<td>160 °C/30 min</td>
<td>0.07</td>
<td>1.83</td>
<td>0.16</td>
<td>0.00</td>
</tr>
<tr>
<td>170 °C/20 min</td>
<td>0.19</td>
<td>2.75</td>
<td>0.21</td>
<td>0.16</td>
</tr>
<tr>
<td>170 °C/30 min</td>
<td>0.47</td>
<td>3.44</td>
<td>0.20</td>
<td>0.43</td>
</tr>
<tr>
<td>180 °C/20 min</td>
<td>0.36</td>
<td>3.26</td>
<td>0.20</td>
<td>0.53</td>
</tr>
<tr>
<td>180 °C/30 min</td>
<td>0.73</td>
<td>4.15</td>
<td>0.35</td>
<td>1.47</td>
</tr>
<tr>
<td>190 °C/20 min</td>
<td>0.92</td>
<td>3.84</td>
<td>0.36</td>
<td>1.44</td>
</tr>
<tr>
<td>190 °C/30 min</td>
<td>1.04</td>
<td>4.28</td>
<td>0.58</td>
<td>1.98</td>
</tr>
<tr>
<td>200 °C/20 min</td>
<td>1.47</td>
<td>4.62</td>
<td>0.31</td>
<td>1.76</td>
</tr>
</tbody>
</table>
and subsequently leads to high glucose yields after enzymatic hydrolysis [38, 39].

However, corncobs were subject to mass loss during SE pretreatment. This effect increased with pre-treatment severities tested and therefore, decreased the recoverable amount of cellulose.

After comprehensive analysis, the most promising pretreatment condition is 190 °C reaction temperature with 20 min retention time (SF = 3.95). This setting causes a favourable intermediate of necessary fibre decomposition, unavoidable mass loss (degradation, evaporation and remaining solids in the SE apparatus) and inhibitor formation respectively.

For a more detailed analysis of an entire biofuel process—a detailed examination of enzymatic hydrolysis, the microbial cell growth, fermentative capacity and an economic and energetic evaluation of every pretreatment condition tested should be done to evaluate the potential of this promising substrate for biofuel applications.

Acknowledgment
The project “Next Generation Biodiesel” (Project-Code: DAABAA_00565) was financed within the scope of the European Union Program “Regionale Wettbewerbsfähigkeit OO 2007-2013 (Regio 13)” from the purse of the European Fonds for Regional Development (EFRE) and the Federal State of Upper Austria, Austria.

References


Investigations on the Thermodynamic Stability and Availability of Nutrients for Plants by Humic Substances Extracted from Peat Samples

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Abstract: This work evaluated the complexation capacity, exchange constants and availability of micronutrients for plants and humic substances extracted from peat samples. Samples of humic substances extracted from two tropical peats (HS-P1 and HS-P2) were enriched with the micronutrients Cu(II), Co(II), Fe(II), Mn(II), Ni(II) and Zn(II) and the parameters for formation of the complexes (HS-N) were evaluated at different pH. The Scatchard model was used to calculate the maximum complexation capacity and the nutrient availability was studied using exchange capacity experiments based on ultrafiltration procedure. The optimum pH for complexation was 4.5 and the order of affinity was: Fe(II) > Cu(II) > Co(II) > Mn(II) = Ni(II) > Zn(II). The maximum complexation capacity reached 56.8 mg g⁻¹ Fe of HS-P1 (the highest) and 1.7 mg g⁻¹ Zn of HS-P2 (the slightest). The exchange experiments showed that HS-P-Fe complexes were formed preferentially. The least stable complex was formed with Zn, which was therefore, more easily available. The results contribute to understand the behavior and availability of some nutrients in soils.

Key words: Peat humic substances, complexation, nutrient availability, exchange capacity, soil.

1. Introduction

Humic substances have undefined chemical structures and can vary widely depending on the characteristics of the environment in which they are formed [1, 2]. The most widely recognized functions of HS in the environment are the complexation and precipitation of cationic species, such as metals and the adsorption of organic compounds [3-6]. These capabilities are due to the functional groups present in the chemical structure of HS, such as carboxylic groups, phenols and ketones, which are responsible for the interactions [7-9]. The interaction of HS with metal species results in the formation of HS-Metal complexes, hence, influence speciation transport and availability of inorganic species in the environment [10, 11].

The beneficial effects of HS in soils include heat retention (due to its dark color), which contribute in seed germination and water retention, which prevents soil erosion. Also HS can be used as a soil conditioner that increases the stability of aggregates, hence, reduces risks of erosion by runoff and decreasing water infiltration rates, which is beneficial in terms of crop productivity [1, 12].

Successful and sustainable food production depends on the quality of the soil, which needs to contain the organic matter and nutrients required for plant growth [13]. HS enriched with micronutrients responsible by plant growth present themselves as an attractive alternative for increasing agricultural productivity. There are some published studies have only evaluated the complexation capacity of elements present as contaminants without considering the natural competition that occurs between elements in the soil [14-16]. The appropriate soil management requires adequate knowledge of the interactions that involve humic substances, which play a major role in the transport, complexation and bioavailability of
Investigations on the Thermodynamic Stability and Availability of Nutrients for Plants by Humic Substances Extracted from Peat Samples

nutrients in the soil. Although this is of fundamental importance in soil chemistry, the literature is fairly sparse in terms of detailed analyses of these processes. Then, the present study investigates complexation and exchange capacity using humic substances extracted from peat samples (HS-P), with the main micronutrients (Cu(II), Co(II), Fe(II), Mn(II), Ni(II) and Zn(II)) required for photosynthesis and plant growth.

2. Material and Methods

2.1 Reagents and Equipment

The stock micronutrient standard solution (100.0 mg·L⁻¹) was prepared from the following salts: CuSO₄·5H₂O, CoSO₄·7H₂O, FeSO₄·7H₂O, MnSO₄·5H₂O, NiSO₄·6H₂O and ZnSO₄·7H₂O. The solution was acidified with HNO₃ and dilute solutions of HCl and NaOH were used to adjust the pH. The solutions were prepared and/or diluted with deionized water (18 MΩ, Milli-QPlus and Millipore) and all materials were decontaminated overnight in 10% (w/v) HNO₃. The micronutrients were quantified by inductively coupled plasma optical emission spectroscopy (ICP-OES), using an Agilent Model 720 instrument equipped with a “seaspray” nebulizer. The instrumental conditions were: RF power of 1.10 kW, argon plasma flow of 15.0 L·min⁻¹ and spray pressure of 200 kPa. Calibration standards were prepared from a 100.0 mg·L⁻¹ stock solution (SpecSol). The detection limits, calculated from the standard deviation of the readings of ten analytical blanks, were (µg·L⁻¹): 3.2 (Cu), 2.4 (Co), 2.3 (Fe), 3.0 (Mn), 1.4 (Ni) and 2.5 (Zn).

2.2 Peat Sampling and Humic Substances Extraction

The peat samples were collected from two peatlands at Sergipe State, Brazil on February 2010, at the 0-20 cm layer, with 5 samples obtained at each sampling point. Both peatlands are distance themselves about 50 km and the differences are little being one with more sand than other. The peat samples were stored in polyethylene bags, air-dried, triturated and homogenized in a porcelain mortar and sieved (2 mm mesh). The humic substances (HS) from the two peatlands (identified here as HS-P1 and HS-P2) were then extracted using the alkaline extraction procedure suggested by the International Humic Substances Society. A peat mass of 300 g was placed in a beaker, which was added 3.0 L of 0.1 mol·L⁻¹ NaOH (1:10 ratio, m/v). The beaker was agitated under a nitrogen atmosphere for 4 h at room temperature (25 °C), using a horizontal shaker which is followed by centrifugation at 2,500 rpm. The humic substances (contained in the supernatant) were concentrated using a rotary evaporator and dried in a ventilated oven at 40 °C.

2.3 Humic Substances Characterization

The two humic substances samples were characterized by elemental and spectroscopic analyses. The elemental analysis employed a Flash EA1112 analyzer (Finnigan Thermo). The carbon, hydrogen, and nitrogen are determined by the combustion method and the oxygen by the pyrolysis method. The spectroscopic analysis was performed by ¹³C NMR with cross-polarization (CP) and magic angle spinning (MAS), using an Avance III 400 MHz spectrometer (Bruker), with a rotation of 5 kHz, a contact time of 2 ms and a relaxation time of 5 s and 11,000 scans. The ¹³C NMR spectrum was analyzed according to the chemical shifts of carbon in different spectral regions [17].

2.4 Determination of Complexation Capacity (CC)

The equilibration time was determined in order to establish the ideal times for the complexation of Cu, Co, Fe, Mn, Ni and Zn with the humic substances. A volume of 10 mL of the stock solution containing 100.0 mg·L⁻¹ of each nutrient was placed in an Erlenmeyer flask together with 90 mL of 100.0 mg·L⁻¹ (w/v) humic substances (HS-P1or HS-P2). The flasks were kept under agitation at ambient temperature (25 °C) and the ionic strength was maintained to 0.1
mol·L⁻¹ with NaNO₃. The analytical procedure proposed by Burba et al. [18] was employed, which is based on a tangential ultrafiltration system equipped with a 1 kDa porosity cellulose acetate membrane (Millipore) (Fig. 1). This membrane prevented the passage of HS and HS-N complexes with a molecular size greater than 1 kDa, hence, only allowing the passage of micronutrients that were not complexed with the HS (or that were free in solution). At predetermined times (10, 20, 30, 40, 60, 90, 120, 240 and 1,440 min), aliquots (2 mL) of the filtrate were collected for determination of the micronutrient concentrations. The experiments were performed in triplicate.

The complexation capacity (CC) of the humic substances with the micronutrients was evaluated at four different pH values (2.5, 4.5, 6.0 and 8.0) in order to identify the conditions that provided the highest degree of complexation. A volume of 100 mL of the 100.0 mg·L⁻¹ HS-P1 or HS-P2 sample solutions were added to Erlenmeyer flasks kept under continuous stirring and with the pH values already adjusted. The ionic strength was maintained to 0.1 mol·L⁻¹ with NaNO₃. Aliquots of 2 mL were then collected from the solutions containing only humic substances (at time zero, prior to addition of the micronutrients). The micronutrients Cu(II), Co(II), Fe(II), Mn(II), Ni(II) and Zn(II) were then added using successive aliquots of 100.0 mg·L⁻¹ stock solution, every 20 minutes (equilibration time), to give final concentrations of 0.1, 0.2, 0.4, 0.6, 0.8, 1.2, 1.6, 2.0, 3.0, 4.0, 5.0, 6.0 and 8.0 mg·L⁻¹. After every addition, aliquots of the filtrates (2 mL) were collected for subsequent determination of the micronutrients. The pH was corrected for each aliquot added of the micronutrient stock solution. The experiments were performed in triplicate.

2.5 Determination of Exchange Capacity (EC)

The exchange capacity of each micronutrient was determined in the presence of the other micronutrients.

Each exchange micronutrient was added to a solution of previously complexed humic substances. The exchange experiments were conducted using Erlenmeyer flasks containing 100 mL of the 100.0 mg·L⁻¹ HS-P1 or HS-P2 sample solutions which was previously complexed with the micronutrients at the highest concentrations found in the CC experiments. The pH was adjusted to the optimum value found in the CC experiments (pH 4.5). An aliquot of the filtrate (2 mL) was collected prior to addition of the exchange micronutrient and this point was taken as zero. Aliquots of each exchange micronutrient (copper, cobalt, iron, manganese, nickel and zinc from an individual stock solution of 100.0 mg·L⁻¹) were successively added every 20 min to give concentrations of 0.2, 0.6, 1.0, 1.4, 1.8 and 3.0 mg·L⁻¹.

4. Results

4.1 Structural Characterization

The elemental composition (carbon, hydrogen, oxygen and nitrogen) results are presented in Table 1, together with the atomic ratios. The H/C and O/C atomic ratios for the HS-P1 sample were lower than for HS-P2. The H/C atomic ratio provides information on the degree of saturation of carbon in an organic molecule, with lower ratios being indicative of greater aromaticity of the HS. Besides, the O/C ratio is
indicative of the carbohydrate content, with a lower ratio reflecting lower carbohydrate content. Therefore, the aromaticity of the HS-P1 sample was higher than that of HS-P2. The C/N ratio provides an indication of the origin of organic matter in natural environments. Values below 20 are indicative of humification processes favored by microbial activity, while values greater than 20 indicate the predominance of plants in the humification process [19]. For both HS samples, the humification process was associated with the presence of plants.

Nuclear magnetic resonance analysis (13C NMR) enables the relative percentages of the different types of carbon present in the structures of the samples to be estimated, so, possible structures of the humic substances can be proposed. Fig. 1 shows 13C NMR spectra for the HS-P1 and HS-P2 samples and provides the values of the relative percentages referring to the different carbon groups present which is obtained from integration of areas in different regions of the spectra. Shifts in the 0-45 ppm region were assigned to alkyl carbons and shifts of methoxylated carbons were found in the 45-60 ppm region. Other features included shifts at 60-110 ppm (alkyl carbon), 110-140 ppm (aromatic carbon), 140-160 ppm (phenolic compounds), 160-185 ppm (carboxylic carbon) and 185-245 ppm (carbonyl carbon) [20, 21]. More specific carbon shifts have been reported previously, such as those at 60-95 ppm (related to O-alkyl carbons) and at 95-110 ppm (related to di-alkyl carbon) [22]. It can be seen from the relative percentages of carbon groups that the shifts related to aromatic, phenolic and carboxylic carbons were more pronounced for the HS-P1 sample. While, the HS-P2 sample showed more pronounced shifts related to aliphatic carbons. This implies that the structure of HS-P1 was more aromatic when compared to HS-P2. This is in agreement with the elemental analysis (Table 1). Since the HS-P1 sample presented a chemical structure that was richer in aromatic phenolic and carboxylic groups, compared to the HS-P2 sample, it would be expected to show different behavior in terms of the complexation process, micronutrient enrichment and nutrient bioavailability.

4.2 Complexation Capacity (CC)

The equilibration time was established by measuring the concentrations of micronutrients free in solution as a function of time after their addition. Equilibrium between the humic substances and the micronutrients (HS-N) was reached in 20 min, after which no changes were observed in the micronutrient concentrations (Fig. 2).

The concentrations of the micronutrients free in solution were plotted against the concentrations complexed to the HS at different pH values. The graphs obtained resembled the adsorption isotherm curves generally obtained from highly adsorbent materials [23]. The behavior obtained from the complexation capacity of HS-P1 and HS-P2 enriched with the micronutrients was studied in four different pH levels. In most cases, exponential curve profiles indicated that the complexation of micronutrients proceeded progressively up to the point where the active complexation sites began to be saturated with the micronutrients. After complete saturation, the maximum complexation capacity could be determined from the inflection point of the curve [24]. For both samples (HS-P1 and HS-P2) and all micronutrients, the best complexation capacity was obtained at pH 4.5.

Once the best complexation conditions have been established, the CC can be calculated using mathematical models [25-28]. The Scatchard model,
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Fig. 2 Equilibrium time for the complexation of micronutrient: Cobalt (-.), Copper (-■-), Iron (-●-), Manganese (-▼-), Nickel (-▲-) and Zinc (-♦-) with peat humic substances samples HS-P1 and HS-P2. (conditions: humic sample concentration 100.0 mg L⁻¹).

which has been used in several previous studies [29-30], considers the complexation process described by Eq. (1) and the stability constant of the HS-N complex can be obtained using Eq. (2).

\[
[M] + [L] \leftrightarrow [ML] \quad (1)
\]

\[
K_c = \frac{[ML]}{[M][L]} \quad (2)
\]

Where, [M] is the concentration of micronutrient free in solution (mg·L⁻¹), [L] is the concentration of ligand (HS), [ML] is the concentration of complexed micronutrient and Kc is the stability constant of the HS-N complex.

Eq. (3) describes the Scatchard model, which represents the relationship between the concentration of the complexed micronutrient (plotted graphically on the x-axis) and the ratio between the concentrations of the complexed micronutrient and the micronutrient free in solution (y-axis) (Fig. 2). For 1:1 stoichiometry, assuming one binding site, a straight line is obtained, with the value of Kc being obtained from the slope and the value of L from the linear coefficient divided by Kc. A graph showing two separate lines is indicative of two or more binding sites, the values of Kc1 and Kc2 are obtained from the linear coefficient divided by Kc1 and Kc2 respectively. The complexation capacity can then be calculated according to Eq. (4).

\[
\frac{[ML]}{[M]} = L \times K_c - K_c \times [ML] \quad (3)
\]

\[
CC = \frac{L}{P} \quad (4)
\]

where, [ML] is the concentration of the complexed micronutrient, [M] is the concentration of the free micronutrient, L is the concentration of the binding sites, Kc is the stability constant of the HS-N complex, CC is the maximum complexation capacity and P is the concentration of the ligand (g·L⁻¹).

Table 2 shows the values of CC calculated for the micronutrients using samples HS-P1 and HS-P2. Sample HS-P1 was able to complex higher concentrations of the nutrients, which could be explained by the structural differences identified from the 13C NMR and CHN analyses. The structure of HS-P1 was more aromatic, containing phenolic and carboxylic groups that increased the complexation capacity. For both HS samples, the order of affinity was: Fe(II) > Cu(II) > Co(II) > Mn(II) = Ni(II) > Zn(II). This order of affinity can be explained by Pearson’s theory, as well as by the Irving-Williams
Table 2  Complexation capacities of the peat humic substances (HS-P1 and HS-P2) for Cu(II), Co(II), Fe(II), Mn(II), Ni(II), and Zn(II).

<table>
<thead>
<tr>
<th></th>
<th>HS-P1 mg·g⁻¹ of HS</th>
<th>HS-P2 mg·g⁻¹ of HS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu(II)</td>
<td>56.8</td>
<td>37.2</td>
</tr>
<tr>
<td>Co(II)</td>
<td>47.5</td>
<td>23.6</td>
</tr>
<tr>
<td>Fe(II)</td>
<td>59.8</td>
<td>57.4</td>
</tr>
<tr>
<td>Mn(II)</td>
<td>35.3</td>
<td>21.7</td>
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<tr>
<td>Ni(II)</td>
<td>32.7</td>
<td>19.4</td>
</tr>
<tr>
<td>Zn(II)</td>
<td>3.1</td>
<td>1.7</td>
</tr>
</tbody>
</table>

series, which considers the stability of the complexes formed with metal ions and takes into account the ionic potential of each metallic element.

4.3 Exchange Capacity (EC)

Once formed, it was important to determine the stability of the HS-N complex in order to evaluate nutrient bioavailability. The stability is normally quite high due to strong interaction of the anionic groups with the metal cations. However, under certain circumstances, the complex can be destabilized as in the presence of another metallic element with higher affinity or due to changes in redox and pH conditions. The exchange capacity provides information about possible changes in the stability of the initial complexes that could occur under different conditions. The exchange constant can be calculated using mathematical models similar to those used for the CC. The simplistic Scatchard mathematical model can be expressed as in Eq. (5):

\[
[HS - N] + [X] \leftrightarrow [HS - X] + [M]
\]  

(5)

Where, \([M]\) is the concentration of the micronutrient, \([X]\) is the concentration of the exchange micronutrient added, \([HS-N]\) is the concentration of the micronutrient M complexed to the HS and \([HS-X]\) is the concentration of the added exchange micronutrient X complexed to the HS. The exchange constant, \(K_e\), is given by Eq. (6):

\[
K_e = \frac{[HS - X][M]}{[HS - M][X]} 
\]  

(6)

The value of \(K_e\) can be obtained from the angular coefficient for the linearized plot of \([M]/[HS-M]\) against \([X]/[HS-X]\).

According to Eq. (6), \(K_e\) is inversely proportional to the stability of the HS-N complex. In other words, \(K_e\) values greater than 1 indicate that exchange of the initially complexed micronutrient by the subsequently added micronutrient is favored.

The \(K_e\) values obtained showed that in the exchange experiments with Cu(II), the complexes of HS with the micronutrients Co(II), Mn(II), Ni(II) and Zn(II) were rather unstable. So, these micronutrients could be easily released (exchanged) to the medium, while, Cu(II) became complexed with the HS. The same behavior was observed for the exchange micronutrient Fe, since it could exchange with Cu, showing that the HS-Fe complex was more stable than any other complex. The two samples of HS showed differences in terms of the degree of exchange, which was generally higher for HS-P1, especially for Cu and Fe.

5. Discussion

Understand the complexation processes involved in the environment is not easy due to the complexity of the composition of all substances present in each environmental compartment. In the soil systems, the processes are influenced by several factors like the solution soil pH, temperature, solar radiation, content of OM, the micro fauna and the concentration of nutrients and others substances with toxic potential [31]. Peat is extremely rich in organic matter and humic substances (HS) is the organic fraction extracted. The structural characteristics of HS are intrinsic to each region, since these are formed by the decomposition of plant and animal residues, which strongly influenced by some factors such as climate, topography and vegetation of the site. Thus, it is important to characterize each HS sample to better understand their composition and therefore, to understand the processes that the HS are involved and operated. The HS extracted from Brazilian peat were
characterized by techniques as elemental analysis and nuclear magnetic resonance of 13C. According to elemental analysis, using the atomic ratios between C, H, O and N, differences can be seen in the two HS samples analyzed. The HS-P1 sample showed a greater content of aromatic C than the HS-P2 sample and therefore, it is expected that the complexation of these samples with the micronutrients will be different. Values of H/C and O/C observed in this study were very similar to those found by Gondar et al. [32] for fulvic and humic acid samples extracted from Spanish peat, where aromaticity was more evident in the humic acids. Fernandes et al. [33] observed values of H/C lower than obtained here for humic substances extracted from highly humified Brazilian peat. This is in agreement with the greater degree of unsaturation (aromaticity) that is observed during advanced stages of decomposition. From this finding, it can be concluded that the HS-P1 sample possibly is in the most advanced stage of decomposition. The results of the NMR analysis confirm the data of elemental analysis, which is evident the shifts from aromatic, carboxylic and phenolic compounds in the HS-P1 sample, while the shifts from HS-P2 sample refer to aliphatic C is more pronounced.

Micronutrients are defined as substances essential to plant development at low concentrations. The main ones that can be cited are copper, molybdenum, iron, boron, manganese chloride, nickel, zinc and cobalt. In this study, it was decided to evaluate the micronutrients that are in cationic form in a wide pH range, such as copper, cobalt, iron, manganese, nickel and zinc. The availability of plant nutrients is influenced by the organic matter content in the soil. Thus, the complexation reactions that involve HS and micronutrients enable to know the dynamics and transport of these nutrients. The process of complexation is influenced by environmental factors such as pH and concentration of organic matter and nutrient. The pH of complexation evaluated in this study was chosen based on the pH observed in Brazilian soils, which can be acid and basic depending on the region in the country. The best pH for complexation of micronutrients with the HS was observed at 4.5. At pH 2.5, the functional groups present in humic substances are likely to be protonated, which hinders the complexation of cationic species due to charge repulsion. In addition, at this pH, micronutrient solubility is high (at the concentrations used). However, at pH 4.5, 6.0 and 8.0, the functional groups present in the HS structure tend to be ionized and are therefore, available for the complexation of cationic species in order to neutralize the electrostatic charges. At pH between 3 and 6, the carboxylic and phenolic groups (major constituents of humic substances) are preferably ionized and therefore, capable of complexing the micronutrients to equilibrate the charges [34]. At pH 6.0, elements such as Fe and Mn can be precipitated and/or hydrolyzed (at the concentrations evaluated), while at pH 8.0, there is further precipitation, with the elements Cu and Zn becoming less soluble in solution. These considerations provide an explanation for the observation that the best complexation was achieved at pH 4.5, since the elements were available in solution and the carboxylic and phenolic groups were preferentially ionized. Garcia-Mina et al. [30] found that the maximum complexation capacity of Cu, Zn, and Fe in HS samples extracted from peat occurred at pH 6-9 due to the greater ionization of the acidic groups present in the HS structure.

From the viewpoint of soil applications, there could be a possibility of soil acidification if the HS-N complexes were applied at pH 4.5. Moreover, López et al. [35] showed that the formation of metal-organic complexes leads to an increase in acidity of soil solution. Nonetheless, acidification can be easily controlled using lime, and silicon has also been shown to provide effective support for the plant [36]. Furthermore, any drawbacks could be compensated by the ability to use a single product containing the micronutrients that are most important to plants as
well as the organic material needed to promote healthy growth.

The Scatchard model was applied to the results of complexation at pH 4.5 to calculate the maximum complexation capacity (CC). And the CC which was obtained confirmed that the HS-P1 sample, which has a chemical structure rich in aromatic, carboxylic and phenolic compounds, interacts with a greater amount of nutrient when compared with the HS-P2 sample. This shows that the knowledge of the chemical structure is important for understanding the processes of complexation and consequently, the availability of the nutrient complexed. Moreover, it can be verified an affinity order for the micronutrients: Fe(II) > Cu(II) > Co(II) > Mn(II) = Ni(II) > Zn(II). According to Pearson [37], all the micronutrients evaluated (with valency of II) are regarded as unreliable or are not even classified as hard or soft acids. However, some studies have shown the ability of the HS to influence the formation of species such as Fe(II) and Fe(III) under certain experimental conditions including solar or artificial irradiation [38, 39]. In addition, Fe(III) is classified as a hard acid and therefore, able to interact with hard bases such as hydroxyl and carboxylic groups. Since humic substances contain phenolic (-OH) and carboxylic (-COOH) groups, they are considered to be hard bases, which explains the stronger interaction with Fe. In the case of the other micronutrients, for which the valency remains unchanged, the Irving-Williams series is applicable: Mn(II) < Co(II) < Ni(II) < Cu(II) > Zn(II), where Cu is the element that forms the most stable complexes [40]. Schnitzer and Kerndorff [41] evaluated the sorption of eleven metals on humic acid and observed a similar behavior being the Fe most sorbed while the Mn was least sorbed. They also verified that the sorption increase with the pH, but some elements like Fe and Cu could form hydroxides at pH 5.8. The results more consistent obtained by them were verified at pH 4.7, which similar to the author’s results.

The stability of the complexes can be changed depending on the environment in which they are exposed. For example, different soil types have different concentrations of nutrients and metal species as aluminum or other contaminants such as lead and cadmium. These substances can act in the complex in case of them which are present in high concentrations or have a greater affinity with the HS. Thus, the need to evaluate the behavior of the nutrients previously complexed to HS against to others substances, allows to know the dynamics of them in the environment.

The exchange capacity experiments showed that the complexes previously formed with micronutrients are altered in the presence of other micronutrients. The HS-Fe complex was the most stable since in the presence of this element (iron), the exchange constants were favored. Araujo et al. [42] assessed the capability for exchange between the elements Al, Fe, Cu and Co. He found that the HS-Al and HS-Fe complexes were more stable than HS-Cu and HS-Co. This behavior was attributed to the valency of the ions. Whereby, trivalent ions such as Al(III) and Fe(III) have less dependency on stability as a function of molecular size. In the case of Zn, in all the experiments it was observed that there was a release of this element in the presence of others. This could be advantageous in certain cultivations like maize, while Zn is a limiting micronutrient for optimal development [43]. The application of HS enriched with a mixture of micronutrients should favor the release of zinc, which would then be available to assist plant growth.

To resume, the complexation processes evaluated in this work showed the pH obtained for optimum complexation was 4.5 and the order of affinity observed was: Fe(II) > Cu(II) > Co(II) > Mn(II) = Ni(II) > Zn(II). The maximum complexation capacity reached 56.8 mg·g⁻¹ Fe of HS-P1 (the highest) and 1.7 mg·g⁻¹ Zn of HS-P2 (the slightest). The difference observed between the two peat humic substances samples were assigned to chemical structure of each sample. The exchange experiments showed the nutrient availability in presence of another
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micronutrient. The HS-P-Fe complexes were formed preferentially. The HS-P-Zn complexes were the least stable. The results support the understanding of the processes of nutrients availability and thus, could help to comprehend how the micronutrients are distributed in the soil.

6. Conclusions

The present study investigated the complexation and exchange capacity of humic substances extracted from peat samples (HS-P1 and HS-P2), with the main micronutrients (Cu(II), Co(II), Fe(II), Mn(II), Ni(II) and Zn(II)) required for photosynthesis and plant growth. The optimum pH for complexation was 4.5 and the order of affinity was: Fe(II) > Cu(II) > Co(II) > Mn(II) = Ni(II) > Zn(II). The maximum complexation capacity reached 56.8 mg·g⁻¹ Fe of HS-P1 (the highest) and 1.7 mg·g⁻¹ Zn of HS-P2 (the slightest). The exchange experiments showed that HS-P-Fe complexes were formed preferentially. The least stable complex was formed with Zn, which was therefore, more easily available. The results contribute to understand the behavior and availability of some nutrients in soils.

Acknowledgements

This research was supported by the following agencies: Fundacao de Amparo a Pesquisa do Estado de Sao Paulo (FAPESP), Conselho Nacional Desenvolvimento Cientifico Tecnologico (CNPq), and Coordenacao Aperfeicoamento Pessoal Nivel Superior (CAPES). The authors are also grateful to Dr. Tiago Venancio (Universidade Estadual Sao Carlos) for assistance with the NMR ¹³C analysis. The authors dedicate this work for Professor Julio Cesar Rocha for his retirement.

References


Investigations on the Thermodynamic Stability and Availability of Nutrients for Plants by Humic Substances Extracted from Peat Samples

Society 1953: 3192-3210.


New Easy Method for the Monitoring of Hg Concentration in Fish, Using a Nanostructured Gold Electrode

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Abstract: The applicability of a gold nanoparticle-modified glassy carbon sensor (AuNPs-GCS) for the determination of inorganic mercury in fresh and canned tuna fish by square wave anodic stripping voltammetry (SW-ASV) is demonstrated. Mercury content in sample Tuna Fish ISPRA T 22 was determined to value the accuracy of the determination. The concentration in this sample is not certified, so, the Hg amount was determined also with atomic absorption spectroscopy (AAS): the results obtained with ASV were in good agreement and confirmed literature value reported for this sample. Then, real samples of tuna fish were analyzed. The voltammetric analyses were performed using previously optimized conditions (deposition potential 0 V, step potential 0.004 V, frequency 150 Hz and amplitude 0.003 V). Medium exchange technique permitted to eliminate possible matrix effects. The concentrations in the real samples were found to be in agreement with the common Hg levels reported in literature for commercialized tuna fish in different countries.

Key words: Gold nanoparticle modified glassy carbon sensor, square wave stripping voltammetry, mercury, tuna fish, medium exchange.

1. Introduction

There is increasing concern about the quality of foods in several parts of world. One source of risk for human health upon consumption of inadequate food is the presence of potentially toxic elements. The availability of reliable procedures for their determination in food is a pre-requisite for studying their effects on humans [1].

Natural global cycling has always been a primary contributor to the presence of chemical elements in the different environmental compartments. In the case of mercury, this process involves off-gassing from the lithosphere and hydrosphere to the atmosphere, where it is transported and deposited onto land, surface water and soil.

Heavy metals are considered the most important form of pollution of the aquatic environment because of their toxicity and accumulation by marine organisms. In particular, mercury pollution has dangerous effect on marine ecosystem and humans. It provokes substantial apprehension because it is a known toxicant. It bioaccumulates and biomagnifies in the aquatic food web and accumulates in fish with unfortunate effects on humans [2, 3].

Therefore, accurate data on mercury levels in food is invaluable in the assessment of Hg exposure risks from food consumption. Exposure to mercury by the ingestion of contaminated water and food products results in mortality, reproductive failure and other health effects in predatory wildlife and humans. The distribution of metals varies between fish species,
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depending on age, development status and other physiological factors. Fish accumulate substantial concentrations of mercury in their tissues and thus, can represent a major dietary source of this element for humans [4].

Most methods for the determination of mercury in fish, fishery products and other biological tissues rely on the use of cold-vapour atomic fluorescence spectrometry (CV-AFS) [5] or cold-vapour atomic absorption spectrometry (CV-AAS) [6]. CV-AFS is not widely available and is expensive, but has lower detection limits and better suited to analysis Hg in small tissue quantities [7].

Electroanalytical techniques appear as attractive alternatives. The availability of sensors for the fast and easy detection of Hg would allow a considerable saving of time and money for the analyses. Furthermore, enable more frequent and widespread food controls. Moreover, electrochemical sensor can be connected to a portable instrumentation that can permit to the operators to make checks on site.

Several types of electrodes have been reported for the determination of mercury by voltammetry and most of the results are well described in the review of Martín-Yerga et al. [8]. In previous papers [9, 10], a new procedure for the determination of Hg and for the speciation of Hg/CH₃Hg using a Gold Nanoparticle-Modified Glassy Carbon Sensor (AuNPs-GCS) without any further modification was described. Gold permits to enhance the pre-concentration effect during the deposition step due to its high affinity to mercury. Thanks to the large surface area of the gold nanoparticles, very low detection limit and with short deposition times, was attained.

Another great advantage offered by AuNPs-GCS is the possibility to work with a renewable active surface which permits to eliminate the problem of irreversible contamination of the gold layer. Thus, minimize memory effects and avoid frequent time-consuming and dangerous mechanical cleaning necessary with solid bulk electrodes.

AuNPs-GCS permitted to quantify mercury concentrations in the low ng/L range with high accuracy and precision. The applicability of the technique was demonstrated determining the Hg concentration in: (i) certified samples: estuarine sediment, BCR 276 and city waste incineration ash—BCR 176; (ii) in real samples spiked with a known Hg concentration—drinking water and vegetables extracts and (iii) in pharmaceuticals—an ocular lubricant gel [10].

In this paper, the attention has been focused the applicability of the AuNPs-GCS on the determination of mercury in the matrix “fish”. Initially, the analytical performance of AuNPs-GCS on synthetic solutions containing known as mercury concentrations were evaluated determining linearity, repeatability, accuracy and sensitivity. Then, the reliability of the technique was tested on a sample of fish known as Tuna fish ISPRA T22: this sample has been adopted by Detcheva & Grobecker [11] to validate analytical procedures and its Hg content is reported in literature. To verify Hg concentration in this sample, an Atomic Absorption Spectroscopy with Graphite Furnace atomizer (AAS-GF) was also used to optimize the instrumental response for this matrix. Then, the applicability of the voltammetric technique to the determination of mercury concentration in commercial fresh and canned tuna fish was valued.

2. Materials and Methods

Digestion of samples was performed in Poly Tetra Fluoro Ethylene (PTFE) bombs with a Milestone MLS-1200 Mega microwave laboratory unit (Milestone, Sorisole, Italy).

Voltammetric analyses were performed with a PGSTAT 10 potentiostat (Eco Chemie, Utrecht, the Netherlands) coupled to a 663 VA Metrohm (Herisau, Switzerland) stand cell. It consists of an AuNPs-GCS working electrode, prepared from a commercial Metrohm GCE (section 2.2.1), a glassy carbon counter
electrode and an Ag/AgCl/KCl (3M) reference electrode. The analyzer was interfaced to a personal computer. The operational conditions were selected and voltammograms visualised and processed with the aid of GPES 4.9.

Analytical grade reagents were used. A 1,000 mg/L standard solution of mercury was prepared from HgCl₂ in 0.012 M HCl. More diluted Hg(II) standard solutions were prepared from the concentrated standards in the supporting electrolyte.

High purity water (HPW) obtained from a Milli-Q apparatus (Millipore, Bedford, USA) was used throughout.

One hundred mg/L stock solutions of HAuCl₄·3H₂O (Sigma > 99.9% trace metals basis) in HPW were prepared and used for the deposition of gold nanoparticles onto the carbon surface.

The characterisation of the sensor surface was performed by scanning electron microscopy (SEM), using an Inspect F with Field Emission Gun LEICA-Stereo scan 410 SEM.

An Analyst 600 Atomic Absorption Spectrometer with Graphite Furnace (AAS-GF) by Perkin Elmer was used to verify Hg concentration in Tuna Fish Ispra T22 sample.

A mixture 1:1 of 1,000 mg/L Pd and 1,000 mg/L Ru (both in HNO₃) was prepared. Then, it was diluted 1:1 with HPW (final Pd/Ru concentration: 250 mg/L) and was used as matrix modifier.

2.1 Deposition of Gold Nanoparticles on the Glassy Carbon Surface

A 100 mg/L HAuCl₄·3H₂O solution (corresponding to 50 mg/L of Au) was prepared in Milli-Q water previously filtered through a 0.45 μm cellulose acetate filter and deaerated by passing a N₂ stream. The GC substrate was polished with a suspension of 0.1 μm alumina in HPW for 1 min. Then, it was rinsed three times with ethanol and water, alternatively and dried using a nitrogen stream. Modification with gold nanocrystals was performed by dipping the GC substrate into the HAuCl₄ solution and applying a potential of -0.80 V for 6 min. The obtained sensor was washed with Milli-Q water and kept in 0.1 M NaOH until use.

Before proceeding with the voltammetric determinations, it was necessary to effectuate an activation step by applying a potential of 0.60 V for 60 s. While, the working sensor was stirred in 0.06 M HCl.

The presence of gold nanoparticles was confirmed by SEM analyses and by cyclic voltammetry. The latter was performed varying the potential from 0 V to 1.3 V to 0 V in 0.5 M H₂SO₄.

When required, the dissolution of the gold layer was performed by varying the potential from 0 V to 1.6 V in 6 M HCl whilst stirring the electrode [9].

2.2 Samples and Sample Pretreatment

Tuna Fish ISPRA T22 was analysed to value the efficiency of the acid digestion and the accuracy of the AuNPs-CGS response for mercury quantification working with this type of matrix. Detcheva & Grobecker [11] reported its application to check the applicability of new analytical techniques for the determination of Hg in fish.

Canned tuna fish, produced in Spain, was purchased in a discount in Torino.

Fresh tuna fish was purchased in a fish shop in Torino Province.

Aliquots of 0.5 g of Tuna Fish ISPRA T-22 were transfer in the bombs and digested without any pretreatment with a mixture of 3 mL of HNO₃ and 3 mL of H₂O₂.

The real samples of tuna fish were previously dried at room temperature and homogenized. Then, aliquots of 0.25 g of obtained samples were treated with the same mixture as the ISPRA material.

The following heating program of the microwave unit was adopted: 250 W for 1 min, 0 W for 1 min, 250 W for 5 min, 400 W for 5 min, 650 W for 5 min and ventilation for 25 min. The bombs were left to
cool at room temperature [10].

The resulting solutions were diluted with HPW to 30 mL for ISPRA sample and to 15 mL for real samples. All the experiment performed in triplicate and blanks were simultaneously run.

2.3 AAS-GF Determination of Mercury

Since the sample Tuna Fish ISPRA T22 is not a “certified material”, the Hg concentration in the considered package was also determined with the aid of AAS-GF. In this way, the obtained results were compared with those reported in literature and verified.

The determination of Hg by AAS-GF was performed following the method suggested by Krata, Jedral & Bulska [12] as a starting point and optimizing it by changing the operating parameters to obtain the best response in terms of repeatability—shape of the signal and stability. The following furnace program was adopted: (1) T = 110 °C, ramp time (Rt) = 5 s, hold time (Ht) = 10 s; (2) T = 130 °C, Rt = 10 s, Ht = 30 s; (3) T = 1,100 °C, Rt = 10 s, Ht = 20 s; (4) T = 110 °C, Rt = 5 s, Ht = 10 s and (5) T = 130 °C, Rt = 10 s, Ht = 30 s.

The technique was previously tested on synthetic solutions containing a known mercury concentration (50 g/L) prepared in the blank (HNO3/H2O2/HPW) obtained by digestion of the reagents as described in par 2.2.

2.4 SW-ASV Determination of Mercury

ISPRA sample: 0.5 mL of obtained solution was diluted to 20 mL with 60 mM HCl in the voltammetric cell. Mercury concentration was determined directly or following medium exchange technique.

Real samples: 0.5 mL of obtained solution was diluted to 20 mL with 60 mM HCl directly in the voltammetric cell. Mercury concentration was determined using medium exchange technique.

After 120 s of deposition at 0 V, a voltammetric scan was performed working in SW-ASV and adopting the following parameters: frequency = 150 Hz, initial potential = 0 V, final potential = 0.8 V, step potential = 0.0040 V; amplitude = 0.03 V; scan rate = 0.6075 V/s and stirring rate = 2,000 r.p.m.

After recording the voltammogram of the sample solutions, aliquots of Hg were added and the corresponding signals were recorded. The standard addition method was adopted for the evaluation of the concentration of mercury in all investigated samples. For concentration, lower than 3 g/L well defined peaks were obtained by subtracting the blank signal from the voltammograms of the sample solutions [9].

Each sample was analysed in triplicate.

Unless otherwise stated, the medium exchange technique was adopted for the analysis. After the electrodeposition step from the sample solutions, the potential was maintained at 0 V with the optional function “Hold” of the instrument. Then, the sample solution cell was replaced by a solution of 0.06 M HCl in which the stripping step was then performed.

After each determination the sensor was maintained in a mixture of 0.2 M HClO4/3 mM NaCl/1 mM NaEDTA for 30 s at 0.80 V to remove residues of mercury from its surface.

3 Results and Discussion

3.1 Gold Nanostructured Active Surface

The formation of the gold nanoparticles on the surface of GC substrate is associated to a color change from black (GC) to red-orange (AuNPs).

The status of the gold nanoparticles deposition was valued with SEM analysis and monitored with cyclic voltammetry.

From SEM image (Fig. 1), it is possible to see the presence of a homogeneous gold layer composed of particles with an average diameter of approximately 100 ± 20 nm. A very good repeatability in the morphology of the nanostructured layer was obtained starting from different GCEs and different brands of Au salts.

A faster and more easily available method to
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monitor the nanostructured surface is CV. Fig. 2 reports the voltammograms recorded in 0.5 M H₂SO₄ with an AuNPs-GCS and for comparison, with a commercial solid gold sensor.

The shape reported in Fig. 2 for the solid gold electrode is well known in literature and identifies a clean gold surface. The anodic peak at +1.25 V is due to oxide formation at gold sensor. The nature of the species formed on the active surface is not well known. The most popular hypothesis is the formation of hydrated oxides or the formation of Au(OH)ₙads [13]. The CV voltammogram obtained with the AuNPs-GCS presents the typical peaks of gold electrodes and a “shoulder” (Fig. 2) before the oxidation peak. Such shoulder is typical for a nanostructured gold surface and it is due to the formation of different multi-oxide species formed on the gold nanoparticles [14, 15].

The cathodic peak at +0.90 V, present with both sensors can be attributed to the reduction of the gold
oxide formed during the anodic cycle. The recorded charge under the reduction peak is generally used for the characterization and monitoring of the electroactive sensor area. In particular, the intensity of the signal is proportional to the amount of deposited Au [16].

As expected, the higher signal obtained by the AuNPs-GCS in comparison with the solid sensor is due to the greater surface area which due to the presence of nanoparticles.

3.2 SW-ASV Determination of Hg in Synthetic Solutions

The performance of the AuNPs-GCS was shown in a previous works [9, 10]. Briefly, the height of Hg peak increased with increasing deposition time a value of 120 s was found to be suitable for concentrations down to 50 µg/L. The repeatability, the linearity, the accuracy, the detection limit of the procedure and the interferences of other cations and anions were evaluated. In particular, in the optimized experimental conditions, very low concentrations of mercury could be quantified with good accuracy. For instance, the concentration measured for a 10 ng/L Hg solution was 9.92 ± 0.05 ng/L. The detection limit was estimated as 0.15 ng/L.

In Fig. 3, the voltammograms obtained during the quantification of 20-60 ng/L of Hg in 60 mM HCl was shown.

3.3 Determination of Hg in Tuna Fish ISPRA T22 Sample by AAS-GF

For the determination of Hg in sample ISPRA T22 by AAS-GF the procedure suggested by Krata et al [12]: (Step1: T = 130 °C, Ramp time = 5 s, Hold time = 10 s; Step 2: T = 150 °C, Ramp time = 10 s, Hold time = 30 s; Step 3: T = 130 °C, Ramp time = 5 s, Hold time = 10 s; Step 4 T = 20 °C, Ramp time = 10 s, Hold time = 10 s; Step 5:1,000 °C, Ramp time = 0 s, Hold time = 4 s and Step 6: T = 2400 °C, Ramp time = 1 s, Hold time = 3 s) was adopted after the following modifications (i) the temperature of atomization was increased from 1,100 °C to 1,300 °C which permits to observe a more define peak; (ii) the hold time during the atomization step was extended to increase the sensitivity and (iii) the pyrolysis temperature was decreased from 450 °C to 250 °C using a suitable matrix modifier (250 mg/L Pd/Ru). The obtained concentration was: Hg = 4.39 ± 0.18 mg/kg, which in agreement with the concentration reported in literature (4.43 ± 0.34 mg/kg), corresponding to a recovery of 99.1%.

3.4 Determination of Hg in Tuna Fish ISPRA T22 Sample by AuNPs-GCS

The applicability of the AuNPs-GCS to the analysis of different types of sample was firstly tested on Tuna Fish ISPRA T22. Table 1 reports the equations of the standard addition curves obtained in two independent analyses, with and without medium exchange, the sensitivity and the recovery, computed considering the literature value as the “true” one.

For ASV analysis, aliquots of the solution obtained after sample mineralization were diluted in the supporting electrolyte (60 mM HCl). In fact, the presence of chloride ions permits to enhance the sensitivity of the mercury signal [17].
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<table>
<thead>
<tr>
<th>Technique</th>
<th>Hg found (% recovery)</th>
<th>Calibration curve /$R^2$</th>
<th>Sensitivity ($\mu$A/$\mu$g)</th>
</tr>
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<tbody>
<tr>
<td>Without medium exchange</td>
<td>4.07 ± 0.52 mg/kg (91.8%)</td>
<td>$y = 0.78 ± 0.04 x + 1.37 ± 0.01$ (µA)</td>
<td>0.78</td>
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<td></td>
<td></td>
<td>$R^2 = 0.999$</td>
<td></td>
</tr>
<tr>
<td>With medium exchange</td>
<td>4.42 ± 0.22 mg/kg (99.8%)</td>
<td>$y = 0.81 ± 0.04 x + 1.47 ± 0.14$ (µA)</td>
<td>0.81</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$R^2 = 0.999$</td>
<td></td>
</tr>
</tbody>
</table>

First of all, the accuracy of the determination of the mercury in the correspondent blank spiked with 0.500 µg/L was tested which obtain a recovery of 99.2%.

As reported in Table 1, a recovery of 91.8 % was obtained analysing ISPRA sample without medium exchange. Probably, some residual components of the sample matrix interfered with the analysis. This difficulty was overcomed by performing the voltammetric analysis using the medium exchange technique. The stripping step was carried out in a solution of 60 mM HCl instead of 60 mM NaCl as reported in our previous work. In fact, in the past, NaCl was used to avoid the formation of aqua regia in cell since some authors supposed that it could damage the Au surface [18]. However, in this manner, a lower sensitivity was obtained. In this study, the possibility to use HCl as supporting electrolyte was valued and it does not cause any problem to Au surface. Using medium exchange technique, the result obtained was in good agreement with the known concentration (recovery 99.8 %) reported by Detcheva and Grobecker who used this and other reference materials to validate a spectroscopic method [11]. The concentration of mercury found by SW-ASV was also in excellent agreement with that obtained by AAS-GF.

3.5 Determination of Hg in the Real Samples by AuNPs-GCS

Since the greater error observed in the quantification of the analyte content in ISPRA sample without the application of medium exchange technique, the other tuna samples were analysed only by using medium exchange.

The voltammograms obtained from the real samples are shown in Fig. 4 and the final Hg concentrations are reported in Table 2, together with the equations of the standard addition curves and the sensitivity observed.

As shown in the voltammograms, the resulting signals are well defined. The blank signal was perfectly overlapped with those of the samples except of course below the mercury peaks. So, it was possible to subtract it and obtain a good baseline to search the peaks.

![Voltammograms](image)

**Fig. 4** SW-ASV voltammograms recorded for from fresh (a) and canned (b) tuna fish samples by ASV after blank subtraction.
### Table 2  SW-ASV determination of Hg in real samples.

<table>
<thead>
<tr>
<th>Tuna fish</th>
<th>Hg found</th>
<th>Calibration curve/R²</th>
<th>Sensitivity (µA/µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canned</td>
<td>0.18 ± 0.005 mg/kg</td>
<td>y = 0.54 ± 0.03 x + 0.31 (µA)</td>
<td>0.54</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R² = 0.989</td>
<td></td>
</tr>
<tr>
<td>Fresh</td>
<td>0.52 ± 0.07 mg/kg</td>
<td>y = 0.51 ± 0.03 x 1.57 (µA)</td>
<td>0.51</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R² = 0.982</td>
<td></td>
</tr>
</tbody>
</table>

### Table 3  Ranges of Hg concentration in canned tuna and in muscle of fresh fish reported in literature [1, 21 and 25-34].

<table>
<thead>
<tr>
<th>Country</th>
<th>Hg (mg/Kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jordan</td>
<td>0.06-0.57</td>
</tr>
<tr>
<td>Brazil</td>
<td>0.025-0.968</td>
</tr>
<tr>
<td>USA</td>
<td>0.01-0.51</td>
</tr>
<tr>
<td>USA</td>
<td>0.053-0.739</td>
</tr>
<tr>
<td>Turkey</td>
<td>&lt; 0.0002-1.14</td>
</tr>
<tr>
<td>Iran</td>
<td>0.01-0.401</td>
</tr>
<tr>
<td>Spain</td>
<td>0.222</td>
</tr>
<tr>
<td>Poland</td>
<td>0.067</td>
</tr>
<tr>
<td>Italy</td>
<td>0.04-1.79</td>
</tr>
<tr>
<td>Libya</td>
<td>0.2-0.66</td>
</tr>
<tr>
<td>Portugal</td>
<td>0.08-1.0</td>
</tr>
<tr>
<td>Persian Gulf</td>
<td>0.043-0.253</td>
</tr>
</tbody>
</table>

The canned tuna contained lower concentration of Hg in comparison with the fresh one. This can be due to the fact that for the preparation of canned tuna, younger fishes are used. In this way, the animals had shorter life time to bioaccumulate Hg in their tissues in comparison with the fish sold as fresh tuna. In the voltammogram of the canned tuna, it is possible to see that another peak at +0.38 V does not present in the case of fresh fish. In a previous study [9], the effect of different ions in solution and among the considered element was investigated and valued: Cu was the only one to give a signal in the considered potential range at +0.38 V which did not interfere with the mercury quantification. The presence of trace of copper in sample solution can derive from the composition of the metallic package as found by Buculei et al. [19].

The European Legislation set a maximum level for heavy metals in fishery products and meat of fish. For Hg, the present limit is 1 mg/kg. The examined samples contained Hg concentrations lower than the admissible value [20].

For canned tuna, the obtained results were compared with literature data [21]. Table 3 shows the concentration ranges generally measured in canned tuna fish commercialised in different countries.

The Hg content in the sample analyzed in this work falls within the commonly reported values. In particular, in bolder face the value of Hg reported for canned tuna sold in Spain is reported, that is very similar to the result obtained in this study for a sample produced in the same country.

For fresh tuna, the results were compared with Hg concentrations recently reported in literature by Perugini et al. [22] and by Olmedo et al. [23]: the former determined the level of Hg in four fish species caught in the Adriatic sea (as the considered sample) and the latter determined mercury concentration in different fish species among tuna. The results are collected in Table 4. Hg level determined in the sample analyzed in the work is in agreement with those reported in the literature.

The amount of Hg that can be accumulated in the tissues of fish is strongly correlated to the size of the specimens. For example, generally Mediterranean yellowfin tuna (Albacore species) contains higher levels of Hg in comparison with some oceanic tuna.
species (as Skipjack), because that the former reach greater size and eat bigger fishes [24].

### 4. Conclusions

In this work, the applicability of the nanostructured electrochemical sensor for the determination of Hg in fish products was demonstrated.

The presence of the gold nanoparticles allows to quantify low mercury concentrations with accuracy and precision and the great sensitivity of the technique permits to dilute greatly the sample solutions.

The use of the medium exchange technique allows to overcome some possible interference effects caused by components present in the sample solution, in particular, when real samples are analysed.

Working with a renewable surface, it is possible to dissolve the gold layer and deposit a new one eliminating the memory effect and possible contamination of the sensor surface.

The findings of this study can be used for the application of electrochemistry in the field of food control and human health protection. In particular, (i) the considerable lowering of the budget required for the instrumentation and the ease of the application would also increase the number of laboratories that could undertake this analysis and (ii) the possibility of connecting this sensor to a portable instrumentation could be permitted to make checks on site.

### Acknowledgements

The authors thank Dr. Rossi and Dr. Imbraguglio for the SEM analyses at Nano Facility Piemonte, INRIM.
New Easy Method for The Monitoring of Hg Concentration in Fish, Using a Nanostructured Gold Electrode


[22] Perugini, M., Visciano, P., Manera, M., Zaccaroni, A., Olivieri, V., and Amorena, M. 2004. “Heavy Metal (As, Cd, Hg, Pb, Cu, Zn and Se) Concentrations in Muscle and Bone of Four Commercial Fish Caught in the Central Adriatic Sea, Italy.” Environment Monitoring and Assessment 186: 2205-2213.


