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Contents

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Assessment of Aquatic Physico-chemistry and Eutrophication Rate at the Lake Tondano

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Abstract: The purpose of this research was conducted to determine the physical factors, namely water temperature, total dissolved solids, total suspended solids, electrical conductivity and chemical water including pH, phosphate, BOD (biochemical oxygen demand), COD (chemical oxygen demand), DO (dissolved oxygen). The factors of physics and chemistry can affect the rate of eutrophication. The data obtained through laboratory analysis of physical and chemical parameters of water samples. Water samples were taken from 8 observation stations. Highly variable results were obtained in each observation station. Data physical parameters were tested with statistical multiple linear regression to determine the effect on water quality, the results show that the physical properties do not affect the rate of eutrophication in Lake Tondano. That is, the amount of total dissolved solids content and total suspended solids, will not affect the rate of eutrophication in Lake Tondano. The results of multiple linear regression satistik test against chemical parameter data that is phosphate, BOD, COD and DO showed as a significant effect on the rate of eutrophication in lakes. The higher phosphate levels will be higher as the increase of the rate of eutrophication in Lake Tondano.

Key words: Physics of water, water chemistry, eutrophication, Lake Tondano.

1. Introduction

Lake Tondano located in Minahasa regency is one of the fresh water natural resources and strategic importance for economic development in north Sulawesi. It can be seen from the function and potential of the lake as a source of food, especially fish and raw material sources of drinking water, hydroelectric energy sources, transportation and attraction [1]. Community activities that stand along the shore of the Lake Tondano is fish farming using floating nets, seines and floating restaurant. This condition is the use of coastal areas and along the lake inlet streams as a determining factor entry of pollutants, such as pesticides from agricultural activities, detergent liquid waste from households, restaurants, and other coliform [2]. If the sources of these pollutants are not controlled, then the decline in water quality and an increase in the rate of eutrophication will continue to be sustainable. Eutrophication is the abundance of nutrients in the lake such as phosphorus and nitrogen which can result in blooming of algae and other microscopic organisms [3-5]. Phosphorus in the sediment can be lifted periodically back from the lake bottom to the surface thus causing eutrophic conditions in aquatic environments [6, 7]. The impact will inhibit

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the entry of sunlight so that the oxygen content required will be reduced in the process of life in the lake, and will also affect primary production in aquatic [3].

Sediment, detergents and the waste dumped in the upstream watershed in the upper part of the river as much as 50 tons per year. Estimated that can be absorbed by aquatic plants as much as 50 [1]. Increased sediment load, which come from the surrounding land erosion and landslides, for rivers and streams can cause a decrease in lake productivity and damage to aquatic environments. Erosion that occurs in lakes and along the Lake Tondano reaches 9% to 45%, which is approximately 12.5 tons/ha/year to 27.6 tons/ha/year [8]. Based on the results of an evaluation study, nutrient phosphorus is dominant in the lake, because it required continuous observation influx of phosphorus into the aquatic environment [5, 9]. Therefore, the purpose of this study was to assess the condition of physical properties and chemical properties of water in the Lake Tondano. Changes in physical properties and chemical properties in the aquatic environment will affect the ecosystem of the lake.

2. Materials and Methods

This study uses survey and field observations and the quantitative approach. The research was conducted in Lake Tondano. Eight observation station locations determined purposively with a consideration of the locations where various community activities such as fish farming, agriculture, farms, lakes tour, culinary tours and settlements. Water samples were taken from 8 observation stations (Fig. 1), namely leleko river estuary, location of floating cages in the village of Eris, in the middle of the Lake Tondano, settlements, panasen estuaries, and lakes area covered water hyacinth. Variables/parameters measured include: temperature (X1), total dissolved solids (X2), total suspended solids (X3), pH (X4), phosphate concentration (X5), BOD (biochemical oxygen demand) (X6), COD (chemical oxygen demand) (X7), DO (dissolved oxygen) (X8) and electrical conductivity (X9). Sampling was done 3 times in eight replicates observation stations, and ex situ measurements. The tools used to analyze samples using Atomic Absorption Spectrofotometer (Shimadzu AA-6300), pH meter (Lutron-pH 211), and UV-VIS Spetrofotometer, DO meter (Thermorussel RL-450). Statistical analysis of data with multiple linear regression method is intended to test the variables that significantly affect the rate of eutrophication in Lake Tondano.

Fig. 1 Map of the study area in Lake Tondano research (2012).

Table 1 Chemical and physical description of the rate of eutrophication of the Lake Tondano waters.

	Parameter/variable	Sampling station								Quality standard (mg/L)
		1	2	3	4	5	6	7	8	
1.	Temp (°C)	25.63	25.03	25.43	25.53	25.97	25.60	25.60	25.63	-
2.	TDS	168.67	162.00	172.00	150.00	139.33	144.00	223.67	166.00	1,000
3.	TSS	18.50	13.27	16.90	12.97	11.50	12.83	21.20	11.80	50
4.	pH	8.38	8.39	7.99	8.07	8.26	8.27	7.74	8.13	6.0-9.0
5.	Phosphate	0.059	0.030	0.047	0.041	0.035	0.034	0.064	0.042	0,2
6.	BOD	4.47	4.90	4.50	3.83	2.90	3.60	4.37	3.00	3
7.	COD	13.26	15.03	8.00	7.50	8.10	8.30	13.10	20.60	25
8.	Electric capacity	0.358	0.339	0.365	0.316	0.294	0.300	0.472	0.352	-
9.	Water hyacinth	60.29	13.62	48.48	40.70	16.24	38.95	67.10	94.72	-

TDS: total dissolved solids, TSS: total suspended solids.

3. Results and Discussion

Results of laboratory analysis of water samples from 8 observation stations in the Lake Tondano waters are presented in Table 1.

The measurement results obtained still meet the water requirements of standards that can be used as a source of drinking water in accordance with the Indonesian Government Regulation No. 82 of 2001 on water quality management and water pollution control. The results showed the water temperature of the Lake Tondano highest at station 5, which is in the middle of the lake, at 25.97 °C. Lowest temperature is at station 2 is the tourist area of the lake at 25.03 °C (Fig. 2).

In the observation of station 7 Panasen River estuary, Fig. 3 showed that the measurement greatest of total dissolved solids is 223.67 mg/L. While at the station 5 is in the middle of Lake Tondano obtained the lowest total dissolved solids of 139.3 mg/L. Total suspended solids in the observation at station 7 which Panasen River estuary.

Total suspended solids of 21.20 mg/L in the observation station 7, is the highest number compared to the other observation stations. While the amount of total suspended solids of the lowest at stations 5 in the central part of Lake Tondano is 11.50 mg/L (Fig. 4).

The first observation station that Leleko River estuary has the largest pH is 8.38, while the lowest pH is at 7 stations estuaries Panasen with pH 7.74. Phosphate levels at station 7, Panasen estuary is 0.064 mg/L which is greater than the observation of the others station. Content lowest phosphate on lake inn tourist sites Sumaru Endo is 0.030 mg/L (Fig. 5). This means that the concentration of phosphate in the aquatic environment is still good, because it meets

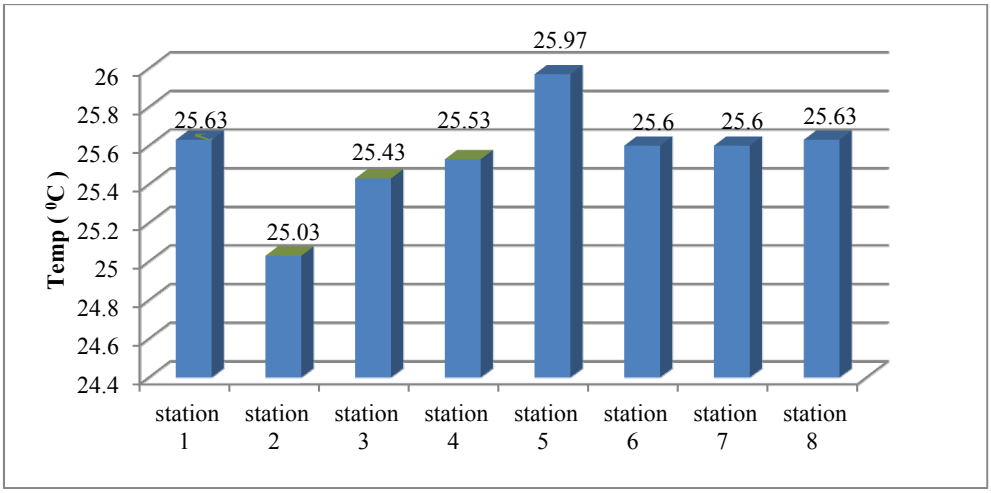

Fig. 2 The water temperature of the Lake Tondano.

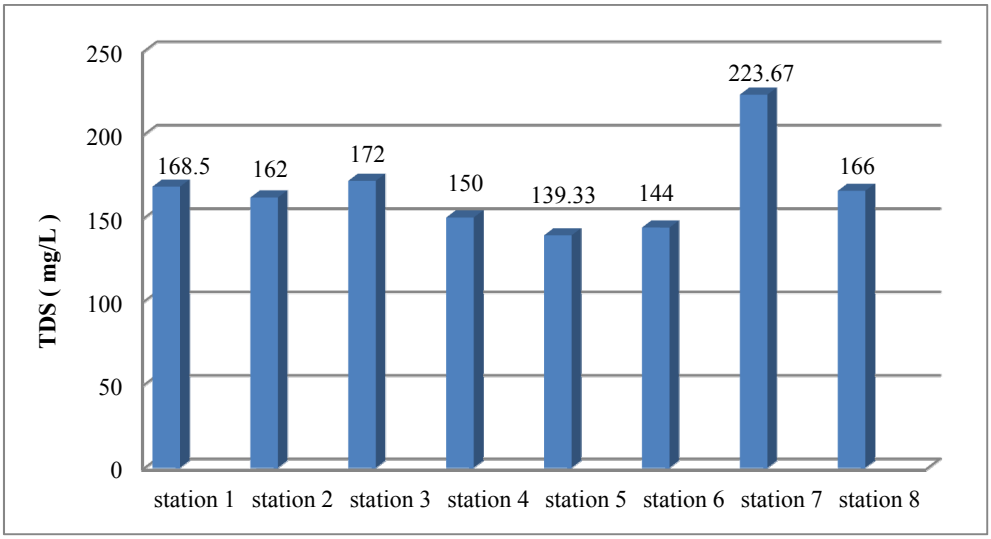

Fig. 3 The content of TDS in Lake Tondano.

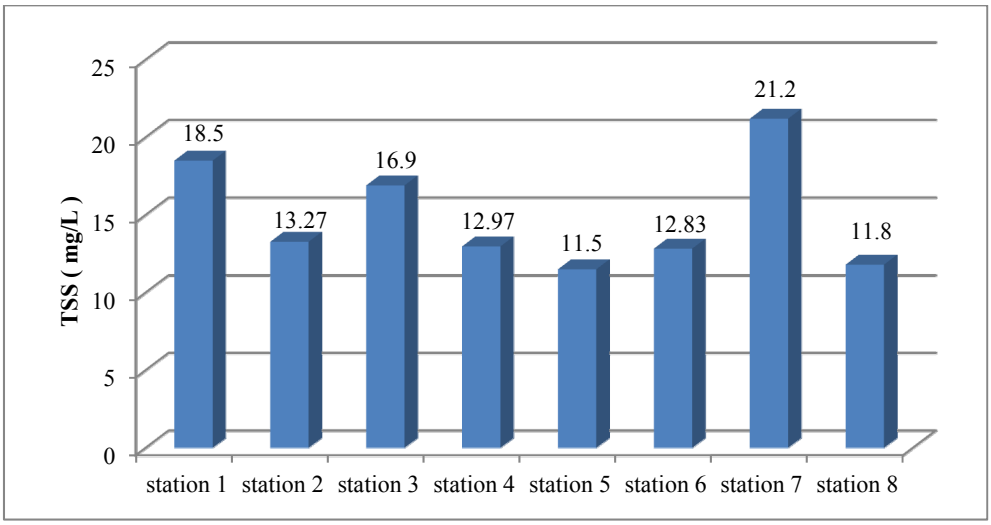

Fig. 4 The content of TSS in Lake Tondano.

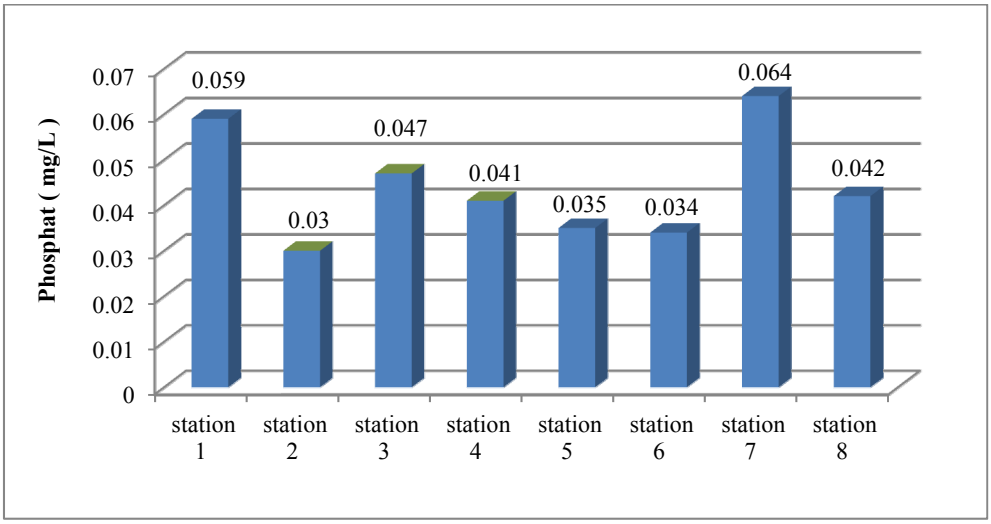

Fig. 5 Distribution of phosphate in the Lake Tondano.

the requirements in accordance with Government Regulation No. 82 of 2001 on water quality management and water pollution control.

Phosphate levels were obtained from 8 observation stations vary widely, the results of the analysis were the distribution of phosphate in the waters of the Lake Tondano be in the range of 30 ug-64 ug PO_4^{3-}-P/L. That is already on the Lake Tondano eutrophic conditions. The indicator can be seen from the extensive closure density at the surface of the water hyacinth in the Lake Tondano (Table 1). Category trophic status of the lake, if the average phosphate levels less than 100 mg PO_4^{3-}-P/L, the status of the lake is eutrophic [10]. Percentage results were obtained on station 1, closing vast water hyacinth in the water level reaches 60%. In the station 7, covers an area of 67.10%, and most extensive are the station 8 is equal to 94.72%. The extent of the surface of the water hyacinth in the smallest at station 2 amounts to 13.62%. Developmental growth of water hyacinth (*Eichornia crassipes*) along the edge of the Lake Tondano can be seen in Fig. 6. Study of Ref. [11] explains that what occurred in south Kalimantan Matasiri Islands waters that phosphorus and nitrogen are elements that contribute to the growth of algae that serve as indicators of water quality and the level of water pollution, if the concentration of phosphate contained in the surface layer of the water and in the bottom of waters is 0.006 mg PO_4^{3-}-P/L, the general meaning is still good water conditions.

When compared with water fertility categories based on phosphate content, the waters of the Lake Tondano include the high fertility rate, because the phosphate concentration obtained at station 7 is a maximum of 64 ug/L. Indicator is the growth of water hyacinth plants that are on the surface of the water can be seen in Fig. 6. Phosphorus greatly affects the trophic condition of the lake water and nutrients that will affect the phosphorus cycle occurs and can lead to the transfer of nutrients in aquatic ecosystems [2, 12]. According to Hoxha, et al. [5], algae growth in aquatic environments can have a negative impact on water quality of the lake. One example is found in the waters of the Lake Tondano (Fig. 6).

Environmental quality standards are used to refer to the measurement of physico-chemical parameters of waters of the Lake Tondano is government regulate on number 82 of 2001, on the Management of Water Quality and Water Pollution Control. The results of the analysis of BOD at station 2, the lake-inn tour Sumaru Endo has the largest concentration of 4.90 mg/L, and the lowest levels of BOD at station 5 in the middle of the lake was 2.90 mg/L. Fig. 7 shows the difference in the content of BOD on each observation station in the waters of Lake Tondano.

Fig. 6 Water hyacinth on the coast of the Lake Tondano.

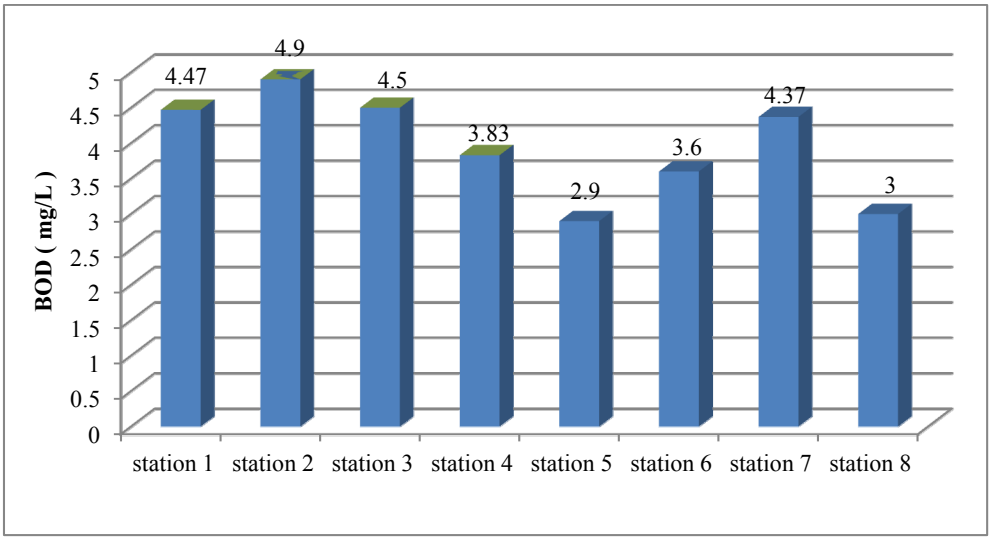

Fig. 7 BOD content of lake waters Tondano.

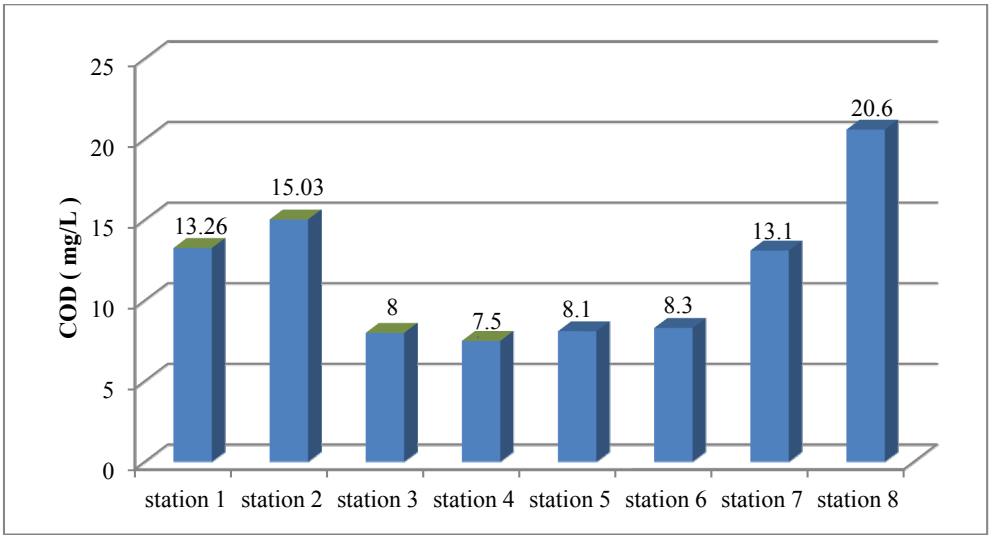

Fig. 8 The content of COD in the Lake Tondano.

Station 8 is the location of the observations of vegetation water hyacinth obtained results of the analysis of COD highest levels of 20.60 mg/L compared to other observation station, the lowest COD levels are at station 4 is the location of floating net cages in the village of Eris at 7.50 mg/L (Fig. 8).

Dissolved oxygen content in the observation station 5 on the central part of the lake sampling sites have the highest levels of DO in the amount of 6.64 mg/L. While the lowest DO levels are in the observation, station 2 is the location of lake-inn tour Sumaru Endo with DO levels 5.67 mg/L (Fig. 9).

EC (Electrical conductivity) in the waters of Lake Tondano varied between observation stations with each other. At station 7 is in the location of the mouth of the River Panasen have EC of which the highest was 0.472 ohm^{-1}/cm. While on station 5 in the middle of the lake has the lowest EC of 0.294 ohm^{-1}/cm (Fig. 10).

Tondano rate of eutrophication of the lake can be seen from the percentage cover of water hyacinth on the surface of the water, the observation station 8 showed extensive water hyacinth closing of 94.72%. While the observation station 2 is a tourist location, Sumaru Endo extensive lake water hyacinth closure was only 13.62% (Fig. 11).

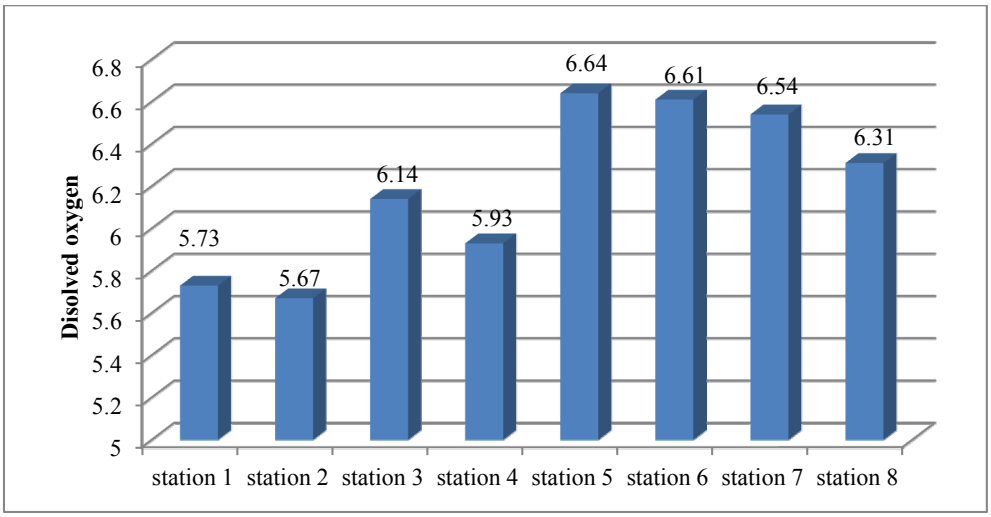

Fig. 9 The content of DO in the waters of the Lake Tondano.

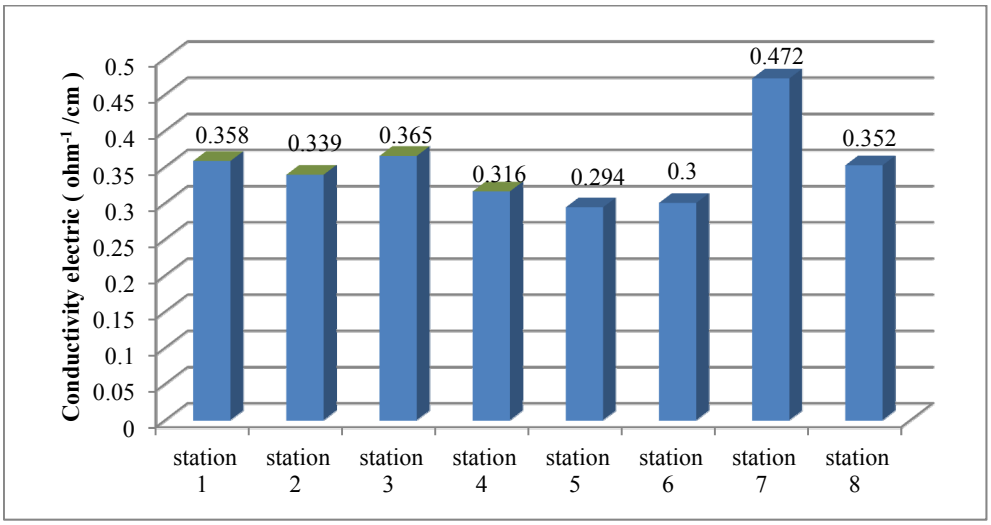

Fig. 10 EC in the waters of the Lake Tondano.

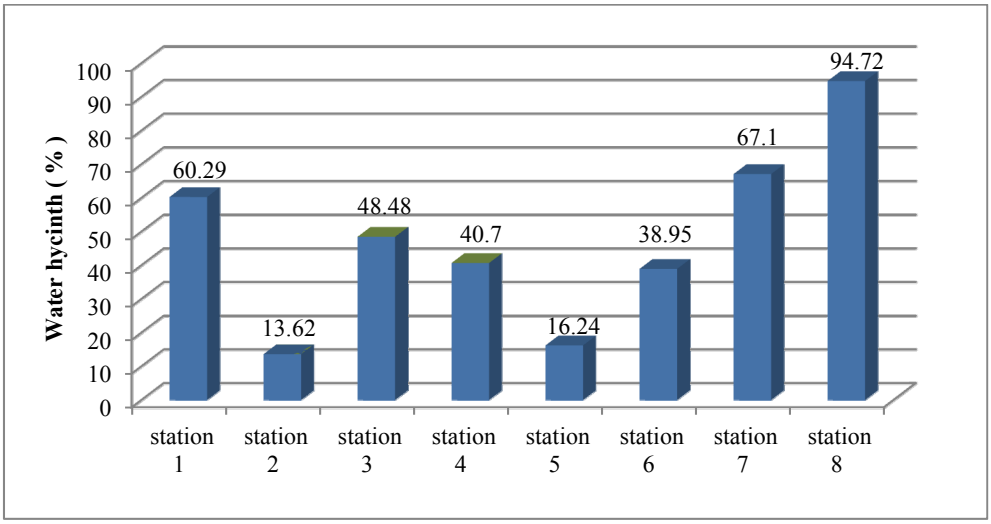

Fig. 11 Percentage of area closures on the surface of the water hyacinth Lake Tondano.

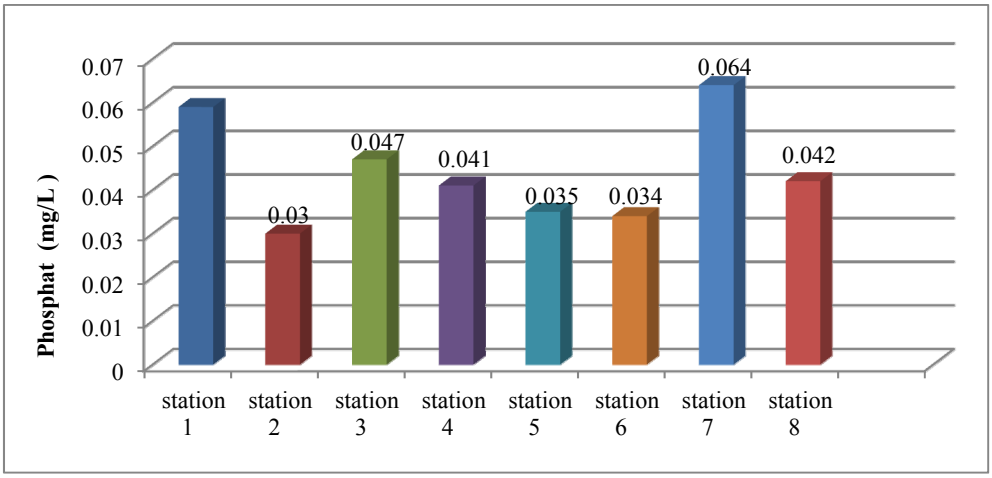

Fig. 12 Distribution of phosphate in Lake Tondano.

The results were analyzed with multiple linear regression model that uses beta coefficient was as following:

$$Y = Bo + B1X1 + B2X2 + B3X3 + B4X4 + B5X5 + B6X6 + B7X7 + B8X8 + B9X9 + e \quad (1)$$

where :

B = regression coefficient;

e = error bullies.

Based on the results of multiple linear regression analysis of the empirical model is obtained as following:

$$Y = 0.296X1 + 0.255X2 + 0.221X3 - 0.234X4 + 0.552X5 - 0.566X6 + 0.711X7 + 0.474X8 + 0.194X9 + e \quad (2)$$

where:

Y = rate of eutrophication;

X1 = temperature;

X2 = TSS;

X3 = total suspended solid;

X4 = pH;

X5 = phosphate Levels.

X6 = BOD;

X7 = COD;

X8 = DO;

X9 = DHL;

e = residual.

The results of the analysis showed that the high content of phosphate in the Lake Tondano waters are significant effect on the rate of eutrophication can be seen in the following diagram Fig. 12.

Therefore, according to E. Sulaksono, et al. [8], it was determined phosphorus as a limiting factor in the lake. The results of observations made along the coast of the Lake Tondano, laboratory analysis and the results of statistical analysis to explain contamination that occurred in the Lake Tondano caused by waste from the activities of coastal communities such as residential, agricultural waste (pesticides), fish waste (fish feed, feces), means of travel (culinary or restaurant, lake tourist), oil and grease from fishing boats and transport boats lake. One of the impact of pollutions that occurs in the Lake Tondano is eutrophication. This happens due to the influx of nutrients, especially phosphorus in aquatic environments. This means an increase in the rate of eutrophication of Lake Tondano which can affect the water quality of the lake, a decrease in water discharge, lake productivity, increased maintenance costs and other hydropower.

Regression analysis showed that there was no influence of physical properties including temperature, TDS (total dissolved solids), TSS (total suspended solids) on the rate of eutrophication in Lake Tondano. That is, the number of TDS and TSS content, will not affect the rate of eutrophication in Lake Tondano. On the other hand, there is the effect of chemical properties include phosphate levels, BOD, COD and DO on the rate of eutrophication in Lake Tondano.

The higher phosphate levels, the higher the rate of eutrophication in Lake Tondano. The rate of eutrophication in Lake Tondano is lowered by lowering phosphate levels, and balance the content of BOD, COD and DO needed in the waters of the Lake Tondano, means need to pay attention and control the entry of nutrients into the lake water phosphorus [15].

4. Conclusions

The concentration of total phosphate contained in some observation stations on Lake Tondano varies, it ranged 30-64 mg PO_4-P/L. Results obtained stating that the phosphate concentration in the Lake Tondano water environment still meet the requirements according to the PP. 82/2001 i.e. water intended for drinking water standard of 0.2 mg/L. According to the results of the regression analysis, the authors concluded that there is no influence of physical properties include temperature, TDS, TSS on the rate of eutrophication in Lake Tondano. On the other hand, there is the effect of chemical properties include phosphate, BOD, COD and DO on the rate of eutrophication in Lake Tondano. The higher phosphate levels, the higher the rate of eutrophication in lakes and that a balance Tondano concentrations of BOD, COD and DO in the water body of the lake.

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Study of Water Injection Efficiency through Modern Geological-Mathematical Models in Guneshly Field

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Abstract: Guneshli is a unique oil field for its huge oil reserves. Fasila layer is one of the main productive series with more than 3,300 tons oil production per year. It is an important task to continue efficently develop this object. Development of Fasila was analysed based on geological-mathematecal models, production performance was predicted through use of advanced "evolution" modeling program. In addition, the impact of water injection from deep water Guneshly was identified and relavant proposals were put forward.

Key words: Productive series, collector, hompers-meychem curves, trend method, evolution modeling.

1. Introduction

Guneshly oil and gas field, located on the Azerbaijan sector of Caspian Sea in the center of Absheron-Balakhany oil and gas zone is one of the largest fields in terms of resources and productivity of collectors. The depth of the sea within the field varies between 80-300 m. 12 km × 4 km Guneshli field has a braxianticlinal form, complicated by tectonic faults. The south-eastern part of the field, stretched in north-west and south-east directions, is operated by AIOC (Azerbaijan İnternational Operating Company). The main production object of the field is productive layer deposits which have been produced since 1980 (Fig. 1).

Fasila layer has the largest oil reserves. Lithology of this group is composed of clay layers of small size as well as of large and medium-size sandstones. Moreover, it has a high collector resistance and a good assortment. Thickness of layer, opened at the depths of 2,700-3,550 m, is about 110-150 m.

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2. Significance of Study

Fasila layer in Guneshly field is under exploitation by two companies. Reservoir is being developed through different development schemes and various injection systems are applied on a field. After performing water injection in DWG (deep water Guneshly) change (increase) in reservoir pressure in SWG (shallow water Guneshly) was observed. Therefore, production wells from that reservoir in the frontier were taken under evaluation. Given the fact that reservoir permeability is high (140 mD-160 mD) in top reservoir, it is essential to study the impact of injected water along with production data through geological-mathematical methods. In order to choose efficient water injection method, suggestions were developed based on below mentioned geological-mathematical models.

3. Results and Discussion

Around 112.6 million tons of oil have been produced from Fasila layer. Paying close attention to the table of the dynamic characteristics of Fasila layer, it can be observed that significant decrease rate in production since 2008 is reduced. Supposedly the

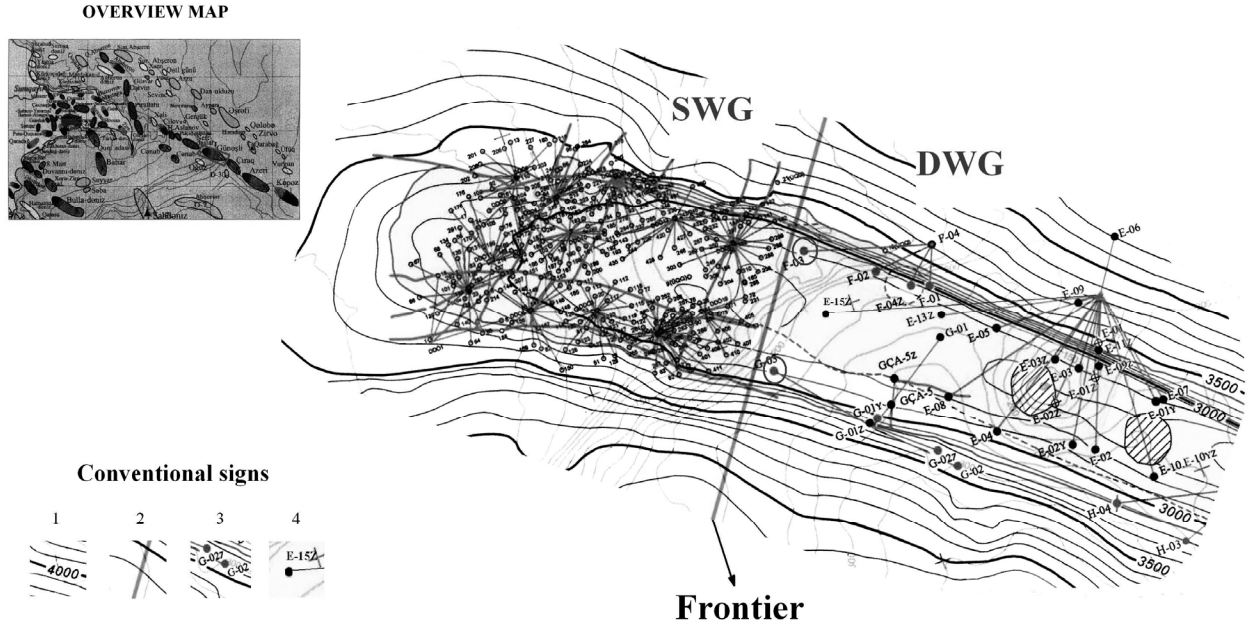

Fig. 1 Map of the Guneshly field. Top of Fasila layer. 1: contour; 2: tectonic faults; 3: water injection wells; 4: production wells.

Table 1 Oil production from Fasila layer.

Years	Annual oil production (Mln. Tons)	The collected oil production (Mln. Tons)	Production falling rate (thousand tons)	Production falling rate (%)
2005	4.001	84.113	-	-
2006	3.936	88.049	65.0	1.6
2007	3.834	91.883	102.0	2.6
2008	3.589	95.472	245.0	6.4
2009	3.579	99.051	10.0	0.3
2010	3.525	102.576	54.0	1.5
2011	3.357	105.933	168.0	4.8
2012	3.372	109.305	-	-
2013	3.301	112.606	71.0	2.1

reason for this impact is water injected from F-03 and G-03 wells in DWG. Thus, in the DWG field total of 12,503 thousand m^3 of water was injected since August 2008.

As seen from the table that the decrease rate in production is reduced every year starting from 2008. This is an indication that the water injected from F-03 and G-03 wells in DWG has a direct effect on production wells in SWG. The correlation can be analyzed by Hompers-Meykem curves on the basis of geological and mathematical models (Fig. 2). For this purpose, Hompers-Meykem curves were drawn using data gathered from many years of oil production in Fasila layer in Guneshly field [1-3]. Annual oil production data from 1980 to 2008 was collected and the dynamic performance was plotted on the graph. Inputting the data into the graph, production prognosis until 2020 can be calculated. However, the graph for 2013 was prepared taking into consideration real production data. In the second case, the predicted collected oil production for the year 2020 additional 9,613 tons was calculated. Paying close attention to the interval from 2008 till 2013 it can be observed that annual oil production until 2010 matches with the prognosis. After 2010, the actual production is above the prognosis. This is proof that water injection in

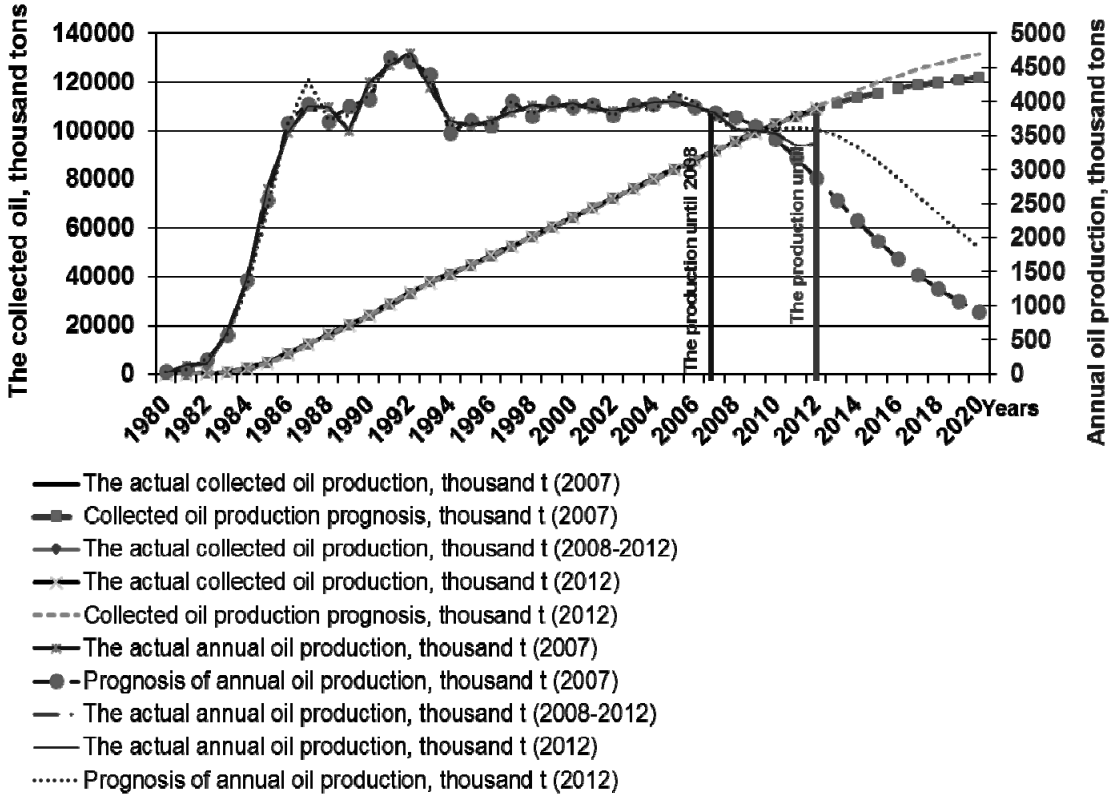

Fig. 2 Hompers-Meykem curves of Fasila layer in Guneshly field.

DWG has an effect on Fasila layer. Thus, although 17,423 thousand tons were predicted to produce, in fact the production for these 5 years were 17,957 thousand tons.

Along with the evolution modeling, other mathematical methods have been used. 9,658 tons of oil was expected to be produced, according to mathematical trend methods, from the wells at the border of SWG regions [4-8]. But the production increase of 11,399 thousand tons of oil were observed as a result of water injection.

4. Conclusions

As a result of water injection, additional 1,741.3 thousand tons of additional oil was produced from the Guneshly field. This means additional 1,755 tons of oil production per day. In 2013 oil production decreased as a result of decrease in water injection. Studies on several wells in the same region show that reservoir pressure increased as a result of water injection (Fig. 3).

5. Recommendation

With the help of "evolution" modeling, mathematical trend methods and the results of the studie, the authors can assume that in order to maintain high and constant oil production it is essential to optimize the volume of injected water along with injection wells across the field.

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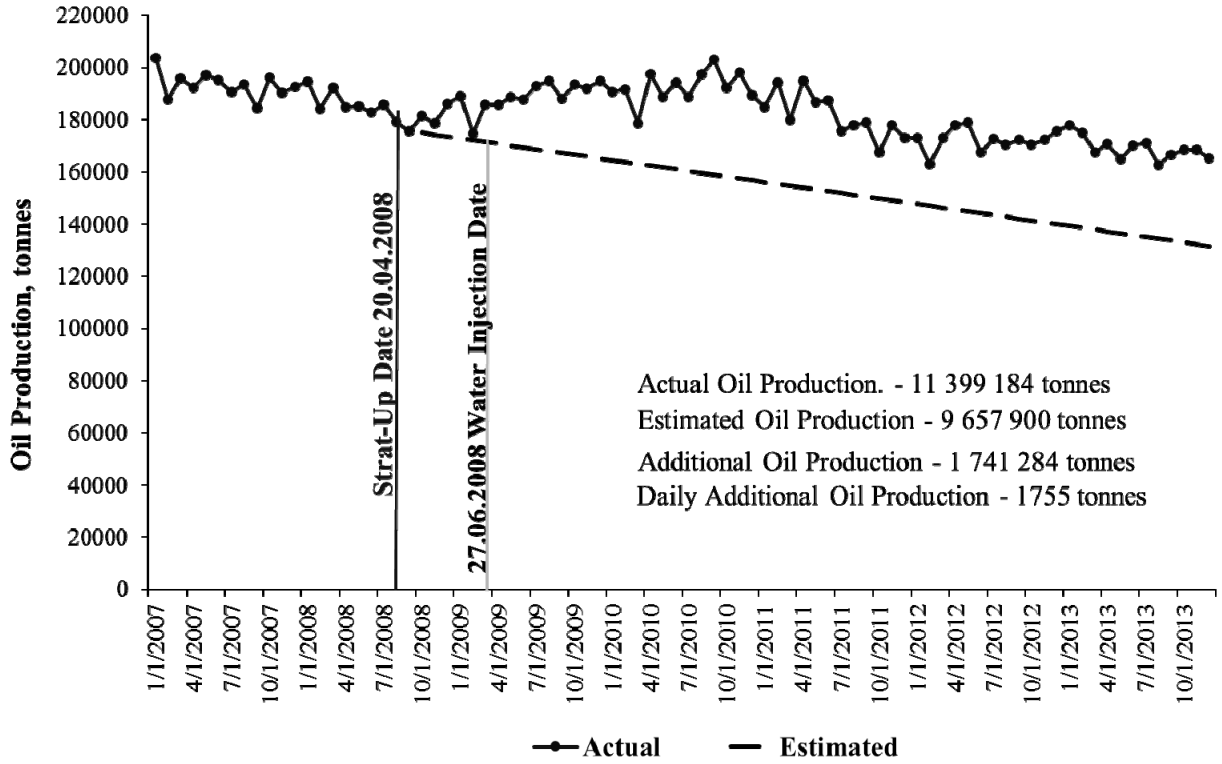

Fig. 3 Trend curves of water injection efficiency.

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Fusarium Vascular Infection of Oil Palm: Epidemiology, Molecular Diagnostic Tools and the Potential of Fusarium Suppressive Soil in Malaysia

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Abstract: Vascular wilt disease caused by *Foe* (*Fusarium oxysporum* f. sp. *elaeidis*) invades the host xylem and causes a devastating disease of oil palm in west and central Africa. This disease has not been reported in Southeast Asia, in spite of long term importation for breeding purposes of African seed and pollen, shown in this laboratory at the University of Bath to be contaminated with *Foe* disease epidemiology was recently studied in plantations in Ghana. Infection mainly occurred in clusters, implying root-root transmission rather than aerial spread by spores. Molecular diagnostic tools has being developed for: (1) rapid detection and quantification of *Foe* in plant tissue, soil, seed and pollen for quarantine purposes in order to prevent transcontinental spread of *Foe*; and (2) to test efficacy of putative disease resistant or tolerant palm genotypes. We have investigated the possibility of *Foe*-suppressive soils in Malaysia in order to explain the non-appearance of this vascular disease there and possibly to reveal other potential biocontrol agents. The explanation as to why Malaysia has not yet attained the disease is likely to revolve around the soil properties, in particular the microflora. This review reported that greater disease severity based on visual symptoms occurred in autoclaved soils and compost than in untreated soils when oil palm seedlings artificially infected with *Foe*.

Key words: Fusarium wilt, epidemiology, molecular tools, Fusarium suppressive soil.

1. Introduction

Fungal diseases of the oil palm can cause very serious losses in production of the CPO (crude palm oil) and CPKO (crude palm kernel oil). Stem and spear-rotting pathogens caused more than 50% mortality in old stands established after coconuts. Because there are 25 years of productive life for oil palms, losses, especially if early on, come to several hundred thousand dollars per hectare [1]. The most important diseases are vascular wilt caused by *Foe* (*Fusarium oxysporum* f.sp. *elaeidis*), BSR (basal stem rot) (*Ganoderma boninense*), red ring disease (*Rhadinaphelenchus cocophilus*), sudden wilt (*Phytomonas staheli*) and spear rot (unknown pathogen) [1, 2]. The threat of bud rot disease caused by *Phytophthora palmivora* also has been documented in Latin America such as Colombia, Ecuador, Surinan and Brazil whereby this disease affecting the productivity of the fresh fruit bunch [3]. In Malaysia, the most serious disease is BSR and it requires an urgent solution. Nevertheless, *Foe* is regarded as a major threat to the Malaysian oil palm industry, even though this disease has not yet been reported in Malaysia or in southeast Asia [4]. Some of these diseases can be very devastating and are directly responsible for retarding development of oil palm cultivation in countries where they occur.

Vascular wilt disease, also known as lemon frond, wilt disease, Fusarium wilt, fusiarose, trachomycose

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and boyomi is the most important disease of oil palm, affecting western and central Africa comprising the Ivory Coast, Ghana, Benin, Nigeria, Cameroon and Congo Democratic Republic (formerly Zaire) [2]. The disease has also been reported in Surinam, Brazil, Ecuador and Colombia [4]. The causal agent of vascular wilt is the soil-borne pathogenic fungus *Fusarium oxysporum* f.sp. *elaeidis*. The pathogen has been isolated from palms suffering vascular wilt and reproduced vascular wilt when artificially inoculated on palm seedlings and thus confirmed its pathogenicity [5, 6]. The pathogen normally invades intact roots of palms, but can invade wounds [7]. It is thought that growing roots contact dead, infected roots or debris containing *Foe* chlamydospores which can survive extreme environmental conditions [8] but can germinate under favourable conditions, such as in response to root exudates [9].

Once inside the palm host the pathogen colonizes the xylem vessels where it can become systemically distributed to all parts of the palm by conidia carried in the transpiration stream [10, 11]. Infection of the xylem and production of microbial polysaccharides, enzymatic breakdown of vessel walls and host occluding defence responses causes water stress, and hormonal imbalance result in severe yield loss and possible palm death [11, 12]. Symptoms of the disease include stunting and wilting of yellowed fronds [13]. Two disease syndromes are observed—acute or rapid wilt and chronic wilt (Fig. 1). Acute wilt disease progresses rapidly and palms die within 2-3 months, the leaves dry out and broken off by wind action [13].

2. Disease Epidemiology

The diseased palms were reported to occur frequently in pairs, thus indicating infectious spread between neighboring palms [14]. This model of tree-tree spread is supported by the statistical occurrence of infected palms in pairs or groups and the greater infection of palms with missing neighbours than those without [15]. Although it is generally accepted that vascular wilt is spread through the soil through palm root contact with dead, infected palm tissue, it has also been determined that *F. oxysporum*

(a) (b) (c)

Fig. 1 Fusarium wilt symptoms in Ghanaian oil palm plantation: (a) Chronic symptoms whereby the older leaves becomes desiccated and the rachis break near the base. The spear in the crown becomes reduce in size and often chlorotic; (b) A mature palm suffers a rapid death through an acute attack, the leaves dry out and die rapidly while still in erect position; (c) Then, the crown snaps off during strong wind.

sporulates on male inflorescences which suggests possible aerial transmission. In fact aerial dispersal plays a role in Fusarium wilt of some other plant species including date palm [16]. Disease epidemiology was recently studied in plantations in Ghana. Infection mainly occurred in clusters, implying root-root transmission rather than aerial spread by spores [17]. The analysis was based on the hypothesis that infected trees are "randomly" (i.e., independently and uniformly) distributed over the affected area being rejected if the observed CE compression/expression) statistic is smaller for a significance level < 0.01 of the values in the reference distribution. In this study, analyses of the data indicated non-random patterns of disease were evident at all assessments in four different plantations. These results suggest that infection from root to root by *Foe* plays a more significant role to establish infection compared with aerial distribution, even though aerial spread by spores has been mentioned whereby previous study has showed that 96 and 36 viable spores m^{-3} of *F. oxysporum* were detected from wilt and non-wilt areas respectively [16].

A model of tree-tree spread is further supported by the report of infected palms in pairs or groups by Ref. [14]. Palms killed by the pathogen become a source of nutrients for adjacent palms and become infected [13]. Thus, clusters of diseased and dead palms were formed and observed expanding into all directions. Clustering disease patterns was also reported during *Phytophthora* epidemics in bell peppers whereby species of *Phytophthora* can be dispersed either in soil, via surface water movement down rows, from rain splash dispersal, by air, or via movement by humans or invertebrate activity [18]. In this study, many *Foe* isolates were obtained for genetic analysis from diseased palms including the presence of *Foe* in 10 per cent of symptomless palms (Fig. 2). This reveals that visual disease surveys do not show the true picture of the level of infection [17].

3. Molecular Diagnostic Tools

Oil palm seeds are the subject of global breeding programme, thus there is a risk of long-distance transmission of *Foe* on contaminated seeds. Previous studies have reported that *Foe* can contaminate the outside of seeds [19] and can exist within oil palm seeds [20]. Moreover, 3% of commercial seed that were artificially infested also showed wilt symptoms, suggesting that seed transmission is possible [21].

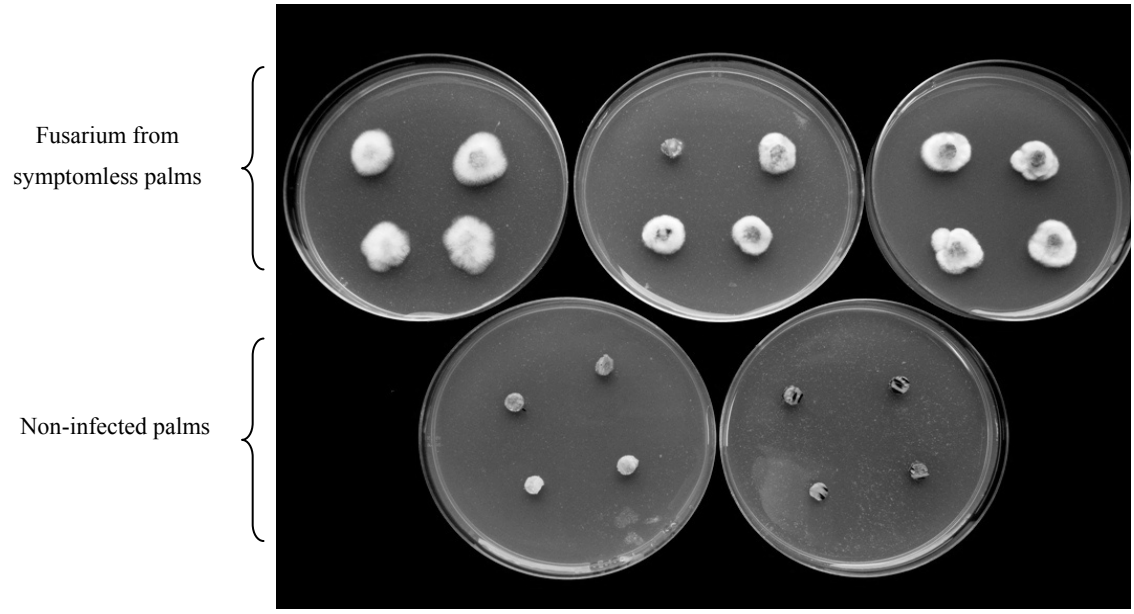

Fig. 2 Re-isolation of *Foe* from symptomless palms on Fusarium selective medium.

They also found considerable variation in levels of contamination between consignments and between individual seeds where about 50% of the consignments were contaminated and levels were as high as 5×10^3 propagules or CFU (colony forming units) per seed; contamination of kernels in 30% of these samples was up to 100 CFU. The source of contamination of pollen and seeds is likely to be the treatment of fruit bunches after harvest, and when *Foe* can proliferate after subsequent retting to remove the pericarp [16].

Contamination with *Foe* of breeding materials remains a major threat to the oil palm industry all over the world. As already described, limited, single plantation outbreaks of vascular wilt of oil palm in Brazil [22] and Ecuador [23] occurred in the 1980s. *Foe* isolates from these south American outbreaks have been shown to have identical RFLP (restriction fragment length polymorphism) patterns to each other and were vegetatively compatible with isolates from the Ivory Coast [24]. Also, further RFLP analysis of a worldwide collection of 76 *Foe* isolates and 21 *F. oxysporum* isolates revealed that isolates from south America had the same restriction pattern as some pathogenic isolates from the Ivory Coast [25]. This evidence suggests the south American isolates originated from African strains and transmission has occurred via exportation of contaminated *Foe* seeds or possibly seeds of a cover crop or on plant debris accompanying the seed, as alternative sources of *Foe*.

3.1 Prevention of Disease Spread: Oil Palm Quarantine

Even though the Malaysia or southeast Asia oil palm industry has enforced strict quarantine regulations on the importation of oil palm seeds in order to prevent the introduction of this potentially destructive disease, the potential of *Foe* to invade the Malaysia oil palm industry is still significant. The demonstration that spores of *Foe* can be carried on oil palm seeds [19] and on the kernel surface inside the shell [20] poses potential problems for plant quarantine. Therefore, prevention of importation of *Foe* to unaffected areas is the most effective control measure as breeding programmes often involve international partners and the continued exploitation of genetic diversity is essential [12].

Oil palm seeds are now subject to strict quarantine regulations, especially where seeds from West Africa are being sent to southeast Asia. The standard dormancy-breaking heat treatment at 40 °C substantially reduces *Foe* infestation but does not eradicate the pathogen [21]. A method of vacuum infiltration of seed with a fungicide, was developed to eradicate *Foe* from the seed coat and from within the seed [21]. Unfortunately a decontamination method for pollen has yet to be achieved. This vacuum infiltration method is used now by CABI (Commonwealth Agricultural Bureaux International) Bioscience in collaboration with the MPOB (Malaysian Palm Oil Board) for all material entering Malaysia and with Indonesian companies. Pollen is less frequently imported but is also screened through re-isolation on Fusarium selective medium and identification using molecular diagnostic tools.

A reliable, robust, and accurate detection method needs to be developed in order to detect the presence of the pathogen, both as spores and as resting thick-walled chlamydospores, in pollen, seeds, soils, and infected palms. Currently, this can be done by morphological identification following culturing on Fusarium-selective medium [26], and using non-destructive sampling by means of removing cylinders of palm stems with an auger. Auger samples should only yield *Foe* because other Fusaria do not have the adaptation to overcome palm physical and chemical barriers and recognition [12]. Unfortunately, these methods of detection are slow and there is a need for a fast molecular probe. Currently tools only exist to diagnose *F. oxysporum* based on morphology and on PCR (polymerase chain reaction) primers designed on the TEF (translation elongation factor) gene [27]. Lengthy artificial inoculation of young oil

palms would then be required to confirm the *Foe*. In most cases, this lengthy procedure is not practical.

Based on current requirements, molecular diagnostic tools has been developed for (1) rapid detection and quantification of *Foe* in seed and pollen for quarantine purposes in order to prevent transcontinental spread of *Foe*; (2) to test efficacy of putative disease resistant or tolerant palm genotypes; and (3) to facilitate epidemiological studies involving palm tissues and soils [17]. Primers were designed based on a virulence effector gene that excluded 70 other phylogenetically closely related Fusarium species from various hosts and origins. The effector proteins, known as *Six* (secreted in xylem) proteins, are limited within to *F. oxysporum* and are thought to distinguish an individual host-specific f.sp. For instance, all *F. oxysporum* f.sp. *lycopersici* strains contain genes encoding for all the *Six* proteins (*Six1*-*Six7*) [28] while only the *Six6* gene homologue was found in Australian *F. oxysporum* f.sp. *vasinfectum* (*Fov*) isolates [29]. The variation of sequence in *Foe* was significant enough to develop a robust molecular identification of *Foe* based on the presence of effector protein. Specific PCR (Polymerase Chain Reaction) markers for *F. oxysporum* f.sp. *vasinfectum* were also developed based on the same technique [29]. Even though the pathotype primers developed are only capable of detecting as low as 4.3×10^4 spores/mL from Fusarium selective medium, the specificity of the primers are proven robust and show inter-lab reproducibility.

4. Fusarium Suppressive Soils

It is very likely that *Foe* has been transmitted to southeast Asia after many years of importation of considerable amounts of breeding material before quarantine measures were put in place, but despite this there are no reports of vascular wilt. The reason why Malaysia and Indonesia have remained free from vascular wilt of oil palm is unknown. Previous study reported that oil palms in Malaysia are highly susceptible to vascular wilt disease when artificially infected by *Foe*, and *F. oxysporum* was isolated from roots of healthy palms in Malaysia [30]. Although thought to be non-pathogenic, some Malaysian strains were reported to cause mild symptoms in susceptible palms [31]. The climate is also conductive of infection [30]. A study by Mepsted [32] led to one theory: Malaysian non-pathogenic strains of *F. oxysporum* were inoculated into seedling roots and prevented subsequent infection by *Foe*. This suggests a natural biological control system in which competition between introduced pathogenic and native non-pathogenic strains in the soil could be the reason why vascular wilt is absent in Malaysia [31]. Recently, it was indicated that disease progress/extent/symptoms of vascular wilt disease in artificially infected palms were substantially delayed/reduced in two Malaysian soils compared to other growth media, highlighting the possibility that *Foe*-suppressive soils in Malaysia might explain the non-appearance of this vascular disease there (Fig. 3) [17].

From this study other potential biocontrol agents may be revealed, for example endophytic fungi that showed antagonism to *Foe* were isolated from plant species grown in Malaysian soils. Endophytes isolated from various plants growing in Malaysian soils showed degrees of antagonism towards *Foe* with 10 out of 15 isolates recording more than 50% inhibition. These endophytes were identified as *F. oxysporum* and *Trichoderma* spp. based on their morphological characteristic and molecular identification [17].

Suppressive soils have frequently been reported to explain reduced Fusarium infection caused by soilbome fungi, even though a strong pathogen and susceptible host are present. Fusarium suppressive soils also can be classified into two types which are the classical type which suppresses only pathogenic Fusarium, and the forest-soil which suppresses all Fusarium isolates [33]. There were many reports on soils that are naturally suppressive to Fusarium wilts

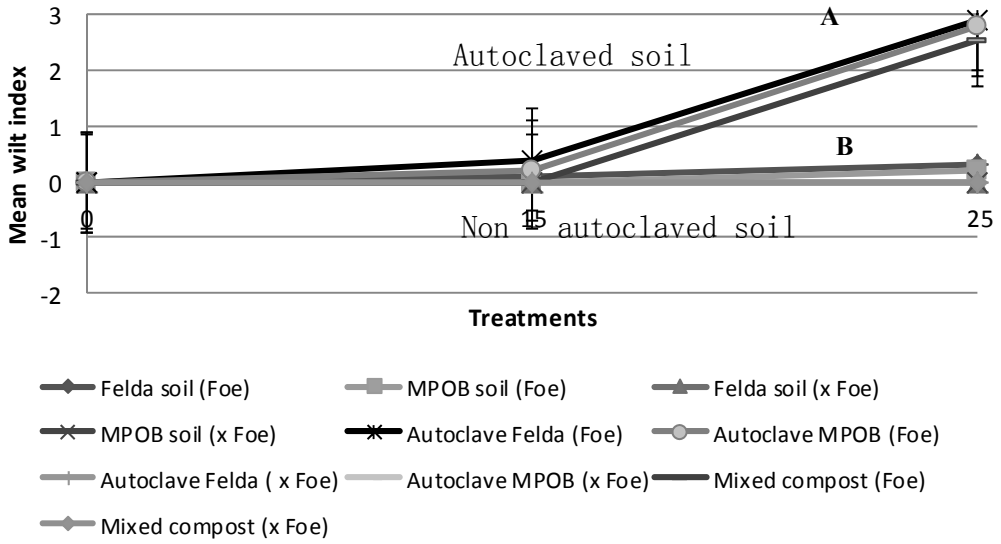

Fig. 3 Wilt symptom development in oil palms inoculated with Foe 16F in sterile and non-sterile Malaysian soils. This data was analyzed by Tukey HSD (Honest Significant Difference).
*Different letters denote a significance ($P < 0.05$) between soil treatments.

of numerous crops are known to occur in many regions of the world [34-36]. Suppressive soils have been characterized for a number of plant diseases including those caused by the cyst nematode *Heterodera* spp. [37, 38] the bacterium *Streptomyces scabies* [39] and the fungi *Gaeumannomyces graminis* var. *tritici* [40], *Phytophthora cinnamomi* [41], *Plasmodiophora brassicae* [42], *Pythium* spp. [43] and *Rhizoctonia solani* [44].

In soils and in soil-less cultures, the application of biological control agents, such as non-pathogenic Fusarium and *Pseudomonas fluorescent*, reduced disease severity and increased yields of different vegetables and flowers [45]. Competition between non-pathogenic *F. oxysporum* (*Fo*47) and pathogenic strain (Fol8) for nutrients rather than for infection site was observed when these fungi were inoculated together in suppressive soil [46]. Also it is possible that limited infection of palm roots could induce host resistance creating "primed" hosts [46]. Previous study showed that the glucuronidase activity of the GUS (*β*-glucuronidase)-transformed pathogen was reduced in the presence of the non-pathogenic *F. oxysporum* protective strain in suppressive soil and concluded that these strains were competing for root colonization [47]. Direct competition between two strains of *F. oxysporum* within the vessels of the host plant was able to reduce the colonization of the carnation *Dianthus caryophyllus* stem by the pathogen, resulting in a decrease in disease severity [48].

Furthermore, some effective non-pathogenic strains of *F. oxysporum* in their capacity to protect plants against their specific pathogens have not only been isolated from soil but from the stems of healthy plants and are presumably endophytic [49]. The presence of antagonists such as species of *Trichoderma,* or other micro-organisms also could give soils suppressive qualities. *T. harzianum* was reported to reduce the germination rate of chlamydospores of *F. oxysporum* in the rhizosphere of cotton *Gossypium* sp. and melon *Cucumis melo* in the presence of *T. harzianum* T35 that was native to suppressive soil and this was attributed to competition for nutrients [50].

5. Conclusions

This study presents new information regarding the disease epidemiology and also development of the first *Foe* specific primers designed as part of

molecular diagnostic toolkit for Foe detection. The occurrence of Fusarium suppressive soils in Malaysia was also explored in this research and one likely contributor, *Trichoderma*, was investigated for potential biological control of *Foe*. Since *Foe* remains as a potential threat to the southeast Asia oil palm industry and Malaysia particularly, the evaluation of current Malaysian oil palm lines for resistance towards *Foe* was investigated. Ultimately, the research progress on vascular wilt disease of oil palm has not only have enhanced knowledge but could contribute to tackling the devastating problems associated with Fusarium wilt that necessitated this research.

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Severe Methodological Deficiencies Associated with Claims of Domestic Livestock Driving Climate Change

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Abstract: Reduction of global livestock numbers and meat consumption have been recommended for climate change mitigation. However, the basic assumptions made to come up with that kind of recommendations reveal severe methodological deficiencies: (1) Carbon footprint, emission intensity, and life-cycle assessments of domestic livestock products reported in scientific literature consistently overlooked the necessity of correcting non CO_2 GHG (greenhouse gas) emissions (nitrous oxide and methane) from managed ecosystems for baseline emission scenarios over time and space (pristine ecosystem and/or pre-climate change emissions); (2) Uncertainties associated with the climate sensitivity of anthropogenic GHG-emissions have been ignored; (3) Inconsistencies in the methodological treatment of land use change (deforestation) in emission intensity calculations (per unit of product) can be detected in the literature; (4) The virtual lack of a discernable livestock signal in global methane distribution and historical methane emission rates has not been acknowledged, theoretical bottom up calculations do not reflect the relative insignificance of livestock-born methane for the global methane budget; (5) Potential substrate induced enhancement of methane breakdown rates have not been taken into consideration. A tremendous over-assessment of potential livestock contribution to climate change is the logical consequence of these important methodological deficiencies which have been inexorably propagated through recent scientific literature.

Key words: Global warming, GHG (greenhouse gases), methane, nitrous oxide, biodiversity, deforestation, baseline scenarios, life cycle assessment, carbon footprint, emission intensity.

1. Introduction

The famous FAO (Food and Agriculture Organization of the United Nations) report "Livestock's Long Shadow" [1] held domestic livestock in general and grassland based production systems in the (sub)tropics in particular, responsible for serious environmental hazards such as climate change, claiming that 18% of anthropogenic GHG (greenhouse gas) emissions are being caused by livestock; more than by the transportation sector. This message produced a storm of incrimination of animal husbandry by the major media around the world. The concern about livestock's alleged contribution to climate change culminated with a hearing in the European Parliament in 2009 on the topic "Less Meat = Less Heat". Few reviews challenged this claim, and those that did received little attention from the media. Pitseky et al. [2] revealed the double standard applied by the FAO in this matter: Whereas a full life-cycle assessment for GHG emissions from livestock products was applied (considering all potential emission sources from land clearing to meat and milk consumption), only fuel consumption was taken into account for the transportation sector.

Nevertheless, even in serious scientific journals authors keep claiming that food consumption patterns potentially contribute to climate change [3-5], an issue still deliberately picked up by prominent media, such as The Economist [6]. Environmentalists recommend a drastic reduction of ruminant livestock at a global scale in order to mitigate climate change and to "yield

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important social and environmental co-benefits" [7]. In another recently published report "Tackling Climate Change through Livestock" [8], the FAO also restates livestock's influential role for climate change, but reduces somewhat the contribution of global domestic livestock to anthropogenic GHG emissions to "only" 14.5%. However, it still:

- contains the same methodological deficits;
- ignores the uncertainties associated with the climate sensitivity of so-called GHGs;
- ignores the inconsistencies between some of its conclusions and several empiric observations in the real world.

2. Methodological Approach

This is a critical review of scientific literature holding livestock responsible for appreciably contributing to climate change. The methodologies applied to come up with such accusations are rigorously assessed in light of:

- lesser known publications not commonly referred to by the mainstream scientific community;
- empirical facts and data determined on a global scale, as well as;
- logical reasoning and rigorous cross checking.

3. Results and Discussion

3.1 How Reliable is the Basic Science on Anthropogenic Climate Change?

The basic assumption which has to be accepted when blaming livestock for causing climate change is noticeable climate sensitivity to anthropogenic greenhouse gas emissions. The overwhelming majority of scientists and scientific organizations (including the FAO) do not question this assumption, given the conclusions of the latest IPCC (Intergovernmental Panel on Climate Change) reports [9, 10] supposedly "unanimously" agreed upon by "hundreds of scientists". However, just focusing on a few critical points, it becomes evident that there is still room for considerable doubt about the above-mentioned assumption of noticeable climate sensitivity to human related greenhouse gas emissions:

(1) Mean global temperatures were flat in the past 15 years, and even slightly decreased over the past 10 years, in spite of steadily increasing CO_2-levels in the atmosphere (Fig. 1) which even caused a remarkable greening of deserts in the past 30 years by fertilizing plants and making them more drought tolerant [11]. This is an empirical observation contradicting all the scenarios of projected temperatures published in the AR4 (fourth IPCC assessment report) and earlier ones. These scenarios are summarized in Fig. 2;

(2) There is an overwhelming number of peer reviewed papers, and among them several published recently such as Refs. [12-19] that acknowledge the existence of various eras after the end of the latest ice age (about 12,000 years ago), which were warmer than or at least as warm as the present age (in spite of the pre-industrial atmospheric CO_2-levels at those times);

(3) Yet, in its TAR (Third Assessment Report), the IPCC [20] prominently featured an iconic graph, first published by Mann et al. [21], in which the temperatures of pre-industrial warm and cold periods, the Medieval Warm Period and the Little Ice Age, had been virtually leveled out, just to show a dramatic temperature increase in the twentieth century. This so called "Hockey Stick" has been exposed to represent scientific bias [22], a serious critique that never has been credibly refuted;

(4) In the AR4-IPCC report, 16 variables are identified as forcing agents of global warming/climate change and are used in the models (however, a number of natural forcing agents are missing). The level of understanding for 11 of them was specified by the IPCC as "very low to low" as shown in Table 2.11 of Ref. [9]. Yet the IPCC comes up with a 90% to 95% certainty in the results of its models, a conclusion which is scientifically incomprehensible, as models based on uncertain variables require empirical validation. As far as the modeled temperature projections for a variety of emission scenarios published by AR4-IPCC

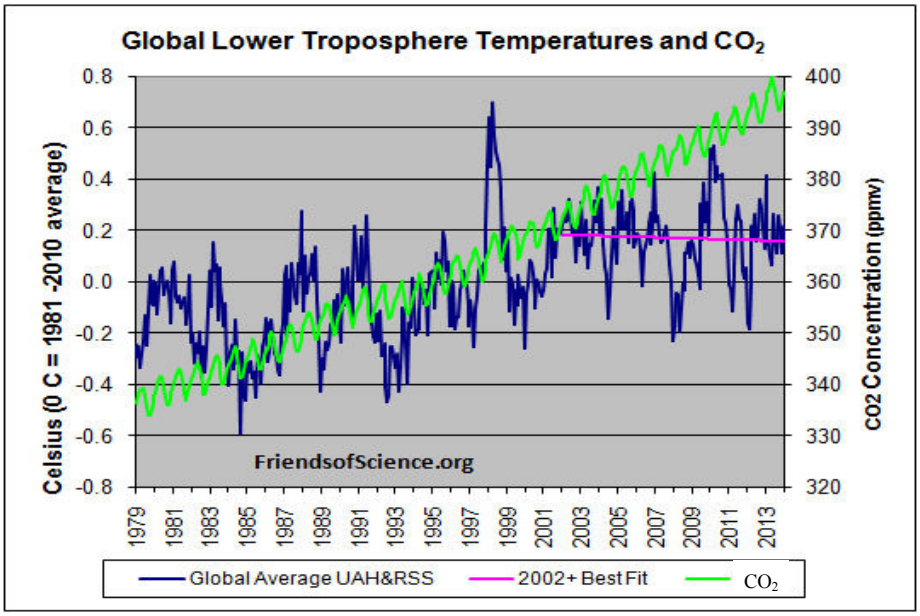

Fig. 1 Observed real world lower troposphere temperature anomalies (average of two analyses of MSU (Microwave Sounding Unit) data from NOAA (National Oceanic and Atmospheric Administration) satellites as processed by UAH (University of Alabama Huntsville) and RSS (Remote Sensing Systems), based on thousands of daily measurements, uniformly distributed over the globe). The best fit line from January 2002 to January 2014 indicates a decline of 0.02 °C per decade. The sharp temperature spikes in 1998 and 2010 are El Niño events. The green line shows the CO_2 concentration in the atmosphere, as measured at Mauna Loa, Hawaii [33].

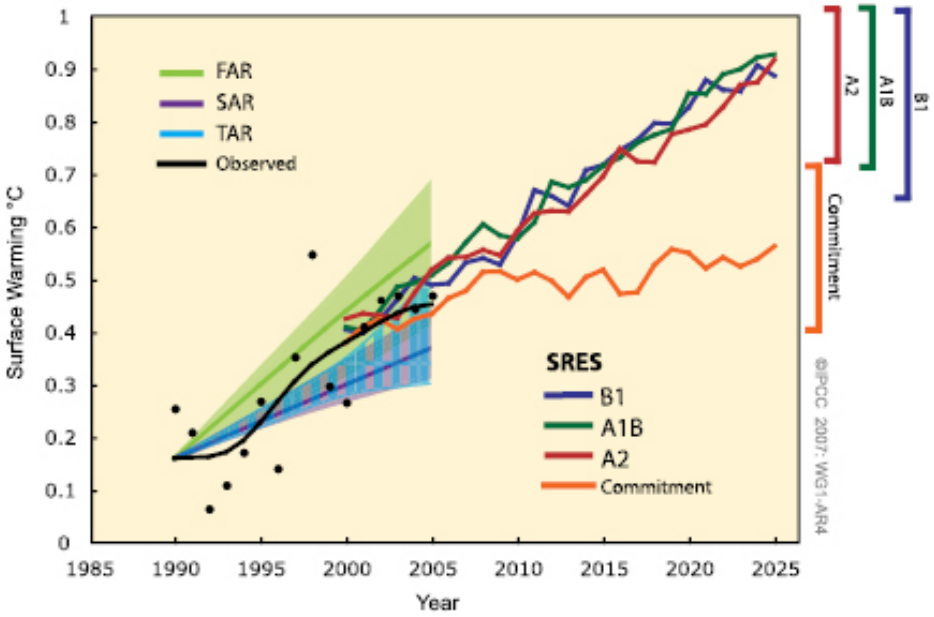

Fig. 2 Multi-model mean projections of global warming for various emission scenarios with uncertainty ranges indicated against the right hand axis, the orange curve (commitment) showing the projection of warming if greenhouse gas and aerosol concentrations were held constant from the year 2000. Projections from earlier IPCC reports, FAR, SAR, TAR (the first, second and third assessment reports), and observed temperatures from 1990 to 2005 are also indicated [9].

(and earlier reports) can already be tested with observed temperature data, recent temperatures are located well outside the confidence intervals of all IPCC-models, which, therefore, did not pass their validation exam as demonstrated in Fig. 1.4 of the leaked second order draft of IPCC-AR5 [23, 24].

However, the IPCC has chosen not to show this graph in the final version of AR5 (the 5th Assessment Report). Additionally, in the Summary for Policy Makers [10] the "observed reduction in surface warming trend over the period 1998-2012" is mentioned on page 15, hidden in the text body and provided with a number of potential explanations. TRCS (The Right Climate Stuff) research team, a volunteer group composed of more than 25 persons, retired NASA (National Aeronautics and Space Administration) Apollo Program veterans (scientists and engineers), also concluded that the IPCC climate models "are seriously flawed because they do not agree very closely with measured empirical data" [25];

(5) The disagreement between models and observed temperature data suggests that the IPCC has largely overestimated within its models the assumed positive feedbacks of the miniscule warming potential of additional CO_2 in the atmosphere [26-28], while in nature negative feedbacks are likely to be in effect [29-31]. The tiny warming of anthropogenic CO_2 in the absence of feedbacks has recently been confirmed by Gervais [32].

3.2 Livestock's Role in GHG (Greenhouse Gas) Emissions

Even if we ignored the above-mentioned objections and kept assuming measurable climate sensitivity to anthropogenic greenhouse gas emissions, there still remain many inconsistencies between empirical facts (and logical reasoning) and the claims of those authors who blame livestock for causing climate change ("Meat = heat"):

3.2.1 CO_2 Emissions and Carbon Cycling

CO_2 emitted by livestock respiration and forage digestion, including the emissions resulting from the consumption of meat and milk, does not increase atmospheric CO_2 levels as it is part of the natural carbon cycle. Not a single livestock-born CO_2 molecule is added additionally to the atmosphere as it has been captured previously from the atmosphere through photosynthesis. The amount of CO_2 released annually by livestock is offset by the photosynthesis of re-growing forages. In fact, long lasting animal products, such as leather and bones, can store carbon for a long period, before it is eventually released again to the air. FAO [1, 8] recognizes the CO_2 neutrality of livestock-born emissions; some other authors (mostly from the popular science and environmentalist arenas) do not with inadmissable arguments [34]: Correct! Domestic livestock is a product of Homo sapiens' survival skills and cultural creative power, but so is CO_2 fixing forage production, pasture cultivation and grassland management. And still, livestock does not exhale a single CO_2 molecule, which had not been captured in the herbage biomass before and which will not be offset by carbon sequestration in re-growing herbage.

The wider natural carbon cycle over geological periods of time, which includes carbonate deposits in the oceans, CO_2 fixation in the lithosphere and fossil fuels, as well as CO_2 recycling to the atmosphere through volcanism and rock-weathering, is not discussed here, although it has been crucial for the steady decline of the CO_2 concentrations in the atmosphere during earth's history, associated with the evolution of highly efficient mechanisms within plants to extract this trace compound, so essential for life, from the air for photosynthesis [35].

The only present-time sources of additional CO_2 emissions caused by livestock husbandry beyond the natural carbon cycle are:

- fossil fuel consumption during the production process;
- deforestation for pasture establishment;
- soil organic matter decomposition from degrading grassland.

These additional carbon sources need to be commented as follows.

3.2.1.1 Fossil Fuel Consumption

Obviously, fossil fuel consumption is considerable when livestock is predominantly grain-fed and held in

confinement (e.g. feedlots). As the annual cultivation of soil is the dominant source of greenhouse gas emissions in primary production [36] and as feed conversion efficiency is lower for ruminants than for monogastric animals, such as pork and chicken [37, 38], grain-fed beef production is a source of considerable CO_2 emissions from the combustion of fossil fuels. In grazing systems, however, the use of fossil fuels is comparatively low or almost negligible, as investments in soil tillage, fertilization, forage harvest and transportation are often marginal or even zero, particularly in (sub)tropical regions. Typically, estimates of greenhouse gas emissions by beef cattle are based on concentrate dominated diets. This provides a considerable distortion of reality when the proportion of grazed herbage increases in the diets, which normally implies a considerable decline in fossil fuel consumption and therefore CO_2-emissions.

3.2.1.2 Deforestation

As far as deforestation for pasture establishment is concerned, what is often overlooked is that this produces one single emission (of 151 t of CO_2 per ha in the average in the case of (sub)tropical South America for example, according to Ref. [39]) at the moment of deforestation or/and shortly after. There is no consistent manner in the literature of methodologically treating CO_2 emissions from deforestation [40] for "life-cycle assessments" (analysis of total environmental impact per unit of a product) or for the appraisal of "emission intensities" (total emissions of GHGs per unit of a product).

The only continent the latest FAO report [8] is blaming for CO_2 emissions from deforestation for pasture establishment is Latin America and Caribbean. South America is charged with the very high "emission intensity" of 100 kg CO_2 equivalent per kg of CW (carcass weight) produced, of which 40 kg CO_2 eq. per kg CW is attributed to deforestation. This is justified with the ascertainment that in other continents there have been no significant deforestations for pastureland expansion recently. However, in other continents, particularly Europe, extensive deforestation took place already centuries ago to establish permanent grasslands.

Mathematically the term "emission intensity" describes the emission of a certain quantity of CO_2 equivalent necessary for producing one kg of a product (in this case carcass) under certain conditions. It is questionable to charge this mathematical term with emissions which are not related to the generation of this particular product. For example, while deforesting a specific area of land, beef production is being carried out on other pasturelands, already established earlier. It is methodologically illegitimate to allot the one-time CO_2 emission from deforestation to any accidentally chosen quantity of a product (e.g. yearly beef production in South America) as is done by the FAO [8].

The single emission from deforestation is generated (and tolerated) in order to produce beef on the new pastureland to be established for a very long period of time in the future (hundreds of years just like on European grasslands). However, when the single "carbon debt" from deforestation is spread over the accumulated production from the deforested area over centuries, the specific emission per kg of product (or "emission intensity") from deforestation tends towards zero.

Therefore other continents such as Europe are treated correctly in the FAO report, by disregarding emissions from "LUC (Land Use Change)". According to the FAO methodological approach, 500 years ago, when there was still ongoing deforestation in Europe (which still has 33% of forested area today, excluding Russia), Europe once reached similar "emission intensities" as South America today (with forests > 47% of its area [41]). And in 10 year-20 year, when deforestation has come to a halt due to legal, environmental policy or physical limitations, emission intensities in South America will be similar to the ones in Europe today. But the FAO report [8] does not tell readers this. Without an explicit footnote

explaining this context, the FAO approach is scientifically dubious. In the tables and figures of the report, values are compared which are not comparable, because they need to be interpreted distinctly and some have (restricted) validity just for the moment. In that way FAO loads (purposely) unrealistically high emission values onto the South American beef industry and onto cattle grazing systems in general. Is this because tropical deforestation reduces competitiveness in the agricultural sector of industrialized countries [42]?

Furthermore it is considered noble and highly ethical to castigate deforestation, particularly in the Amazon, in order to mitigate climate change and loss of biodiversity. However, in the semiarid Chaco of Paraguay, we can show that deforestation for pasture establishment diversifies the habitats and therefore promotes species richness (as demonstrated in gráfico 1 of Ref. [43]), provided the legal land use restrictions of preserving almost 50% of the surface area of each farm in pristine condition (in the form of a nature reserve, bush corridors and islands) are respected, as they are by >90% of the land owners. The additionally created habitats and resources are extensively used by wildlife too. These refer to the bush border effects over many kilometers, savannah-like landscapes, nutritious pastures and rain water collection reservoirs [43, 44].

Part of the "carbon debt" produced at the moment of deforestation is amortized by the considerable amounts of carbon captured and stored in the soil under pastures. This refers to deep rooting tropical grasses [45] and legumes, such as the planted forage shrub *Leucaena leucocephala* [46], and even to spontaneous bush encroachment, which is undesirable in pastures and needs therefore constant control, but stores quite some carbon in the ligneous organic matter kept in steady state equilibrium between vigorous regrowth and decomposition. Furthermore, occasional fires in grass and woodlands also can contribute to Carbon storage as a fraction of the wood, which is burned, is converted into charcoal representing a stable carbon pool in the soil [47, 48].

3.2.1.3 Soil Degradation and Overgrazing

Pasture degradation due to overgrazing is not an inherent characteristic of grazing systems. It is, however, more frequently observed on communal grazing lands in (semi) arid regions than on privately owned lands [1, 49]. Under the constellation of public land and private livestock ownership, there is little interest in private investments in land rehabilitation. Whereas the conversion of native forest into pasture may increase carbon stocks in soil under certain conditions [50], poor management of pastures leads to a reduction of soil carbon [51]. Well managed grasslands are stable ecosystems with no net CO_2 emissions and considerable C storage capacity [52, 53]. In the United States of America, the observed slow decline in the grazing land base in the past decades was generally offset by slight increases in rangeland health and advances in grazing technology [54]. Also in other countries, like Argentina, Australia, and South Africa, there were, anecdotally, positive rangeland health trends during the past century as improved range management practices became more widely used.

3.2.2 Methane Emissions and Cycling

Methane is an atmospheric constituent of only 0.00018% (vol.) which is less than 2 molecules in a million. Just like CO_2, methane emissions also form part of a natural cycle. Oxidation by OH radicals in the atmosphere (modulated by solar ultraviolet radiation, air humidity, and tropospheric ozone) and decomposition by aerobic methanotrophic bacteria in the soil are the major identified methane sinks [55], bringing about a relatively short atmospheric methane lifetime of 8.7 ± 1.3 years according to the IPCC [9]. Recently, Sundqvist et al. [56] showed a net uptake of methane by green plants (both, in the field and in the lab). They suggest that the omnipresence of bacteria with the ability to consume methane could be a possible explanation for their observations. These

results show that plant canopies are playing an important (and hitherto unknown) role as a sink in the global methane cycle.

Methane consumption by methanotrophic bacteria can be considered an autocatalytic process [57], where an enhanced substrate (methane) concentration stimulates the "catalyst's" (bacteria) multiplication. Also, in the case of chemical methane breakdown in the atmosphere to the final products H_2O and CO_2, the reaction velocity (as a general rule of chemical reactions) strongly depends on the concentration of the reaction participants, methane and OH radicals (particularly because their atmospheric concentrations are at a very low order of magnitude). Quirk [58] showed that El Niño induced higher air humidity, associated with an enhanced density of OH radicals consistently increased the sink for methane (i.e. accelerated methane breakdown rate). A similar effect is to be expected with an increased atmospheric methane concentration (while more difficult to demonstrate). Therefore, any change of emission rate also modulates the rate of methane degradation producing a new atmospheric methane concentration as a consequence of a new steady state equilibrium between sources and sinks. On the other hand, a constant emission rate does not change methane concentration in the atmosphere as it is counteracted by a constant or oscillating rate of break down.

Consequently, a constant global livestock number does not increase atmospheric methane, in spite of continuous emissions from ruminant enteric fermentation and manure management. Only an increasing global livestock number could eventually bring about a new steady state equilibrium at a slightly higher atmospheric methane concentration. Therefore, pre-climate change baselines of methane emissions from livestock and global livestock numbers have to be taken into account when assessing the potential impact of domestic animals on climate change. There is, however, quite a bit of dissent on the question of when modern, GHG-induced climate change began (1850 or 1970 or 1990 or not at all?) and with what respective figures. Even the methane emissions by the additional livestock reared after the date assumed to be the beginning of climate change cannot simply be added up over time. Those emissions need to be corrected by the expected enhanced methane decomposition rate, just taking into account the difference of the steady state atmospheric methane concentration built up since that cut-off date due to emissions from the additional domestic livestock.

To determine net anthropogenic emissions from an agro-ecosystem, it is also necessary to correct the measured methane emissions for the baseline emissions which would occur anyway in the natural ecosystem, which meanwhile has been replaced by a managed ecosystem at the very same location. This principle also applies for other non CO_2 GHGs, such as nitrous oxide (Section 3.2.3). Areas formerly populated by large herds of wildlife or areas comprising wetlands, drained later on, could emit even less methane after a land use change towards pastoral land for livestock grazing than did the pristine ecosystem. Moreover, further correction is needed for in situ degradation of methane emitted by livestock, as certain pastoral ecosystems may represent a net sink and not a net source for methane [59], another empirical observation which reduces the utility of bottom-up calculations of methane emissions without considering the eco-systemic context of methane cycling.

Neither FAO [1, 8], nor WWF (World Wide Fund for Nature) [38], nor Vries and Boer [37] in their review of scientific life-cycle assessments for animal products, nor the IPCC [60] in its "Guidelines for National Greenhouse Gas Inventories" consider baseline scenarios over time and space for methane or nitrous oxide. Nor do these publications carry out any corrections for in situ methane degradation, taking into account the sinks counteracting livestock-born methane emissions. They consistently interpret all the direct and indirect emissions of methane (and nitrous

oxide) from livestock or from managed agro-ecosystems at a 100% level as an additional anthropogenic source of GHG-emission (along with fossil fuel-born CO_2). Baseline emissions are treated as if nonexistent. A tremendous overestimation of anthropogenic emissions is the obvious consequence of these simplified bottom-up calculations found in the literature, supposedly scientific. Herrero et al. [5] also repeat these fundamental errors in their otherwise comprehensive review on "biomass use, production, feed efficiencies, and greenhouse gas emissions from global livestock systems".

3.2.2.1 Historic Methane Emissions and Livestock

As shown in Fig. 3, the growth rate of atmospheric methane began slowing down in the early 1980s. The mean methane concentration even topped out for a few years around the change of the millennium, just when cattle husbandry was expanding at a considerable rate, particularly in South America. Between 1990 and 2007, when mean atmospheric methane concentration stabilized completely, the global cattle and buffalo population rose by more than 125 million head, or 9% [61]. This empirical observation is hardly consistent with a domestic livestock contribution to the anthropogenic methane emissions of 35% to 40% as claimed by Steinfeld et al. [1] on the basis of theoretical bottom up estimates. On the other hand, the IAEA (International Atomic Energy Agency) [62] acknowledges domestic livestock being a minor player within the global methane budget because of the poor concordance between global livestock numbers and mean atmospheric methane concentration.

Historical increases of methane concentrations in the atmosphere are best explained by rising fossil fuel extraction and use, as well as the associated technological quality standards, taking into account the considerable gas leakages from older pipeline systems [63]. Also, the stabilization of methane emissions in the 1990s is very likely to be linked to technological changes in fossil fuel production and use. This was suggested by Aydin et al. [64] on the basis of the analysis of ethane as an indicator of fossil fuel-born emissions, detected in air enclosures in firn ice from Greenland and Antarctica. The replacement of leaking pipelines by high quality modern ones from 1970 on in western countries and during the 1990s in the former Soviet Union (particularly in Siberia) best explains the drastic decline of methane growth rate (to even below zero, Fig. 4) towards the end of the last century.

Since about 2008, atmospheric methane has been rising again, but only at about half that of the pre-1990 rate. Quirk [58] suggests that this recent increase of methane emission rate is largely due to natural atmospheric changes modulated by El Niño, La Niña

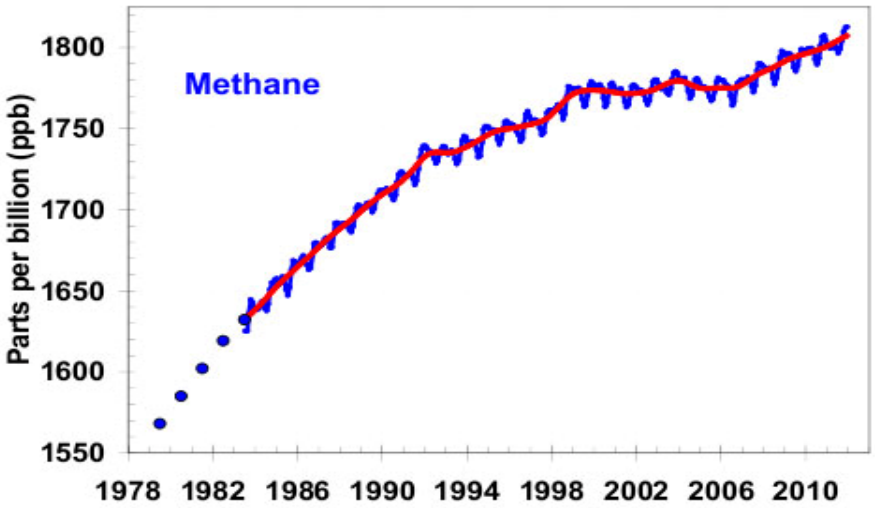

Fig. 3 Mean global atmospheric methane concentration [65].

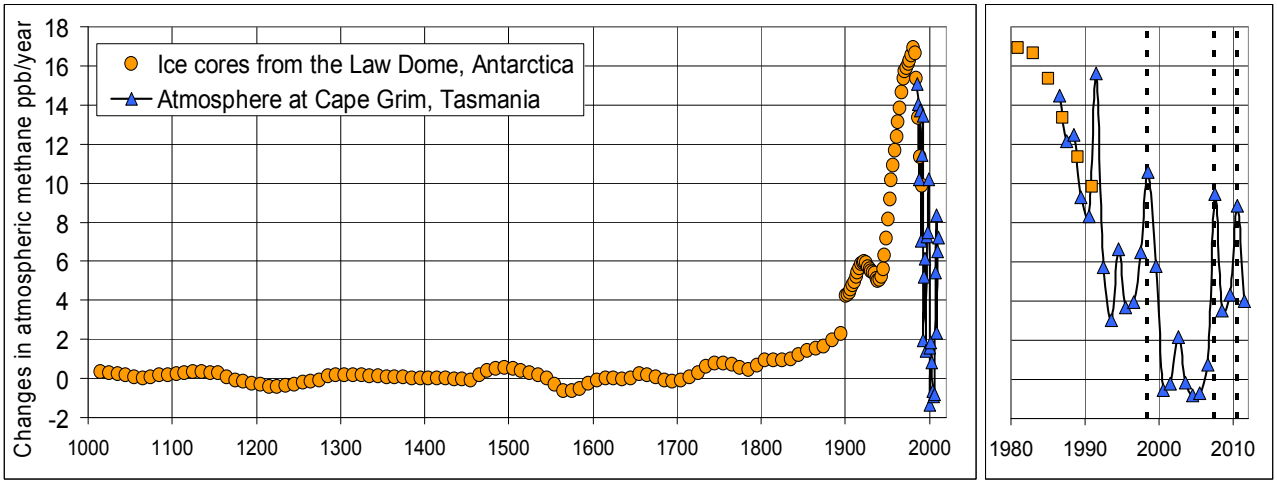

Fig. 4 Annual changes in atmospheric methane in parts per billion derived from ice core up to 1990 and direct atmospheric measurements at Cape Grim (Tasmania) from 1983 to 2011 AD. The peaks in the direct atmospheric measurements reflect the influence of El Niños. The peak in 1991 is an indirect effect from the eruption of Mt. Pinatubo in June 1991 and the 1998, 2006 and 2010 El Nino's are marked by dashed lines [66].

and volcanoes. El Niños enhance absolute air humidity and therefore OH radical density, and volcanoes emit high quantities of SO_2 which competes with CH_4 for OH radicals, thus slowing down methane breakdown. No livestock signal is discernible in the annual variability of atmospheric methane.

3.2.2.2 Geographical Distribution of Livestock and Methane

Contrary to the frequently repeated claims of ruminant enteric fermentation and manure disposal contributing considerably to global methane emissions [1, 5, 39, 67, 68], based on theoretical bottom up calculations, global data show no discernible relationship between atmospheric methane concentrations, as measured by the European satellite ENVISAT over three years, and livestock distribution (Fig. 5): There are regions which are highly populated by livestock with very low (NE-Argentina, Uruguay, Victoria-Australia) and very high (southern China) mean atmospheric methane concentrations, and there are regions with hardly any livestock and very high (parts of Siberia and Amazonia) or relatively low (Sahara) methane concentrations, respectively. Highly populated India, which represents the subcontinent with the highest cattle and buffalo density worldwide, is characterized by moderate methane concentrations. According to the global methane distribution, particularly strong emitters seem to be wetlands in Siberia, humid tropical forests, and paddy rice fields in China. Bottom up estimates of methane emissions from livestock are strikingly different from the atmospheric methane concentrations found in the real world. This allows only one conclusion: Livestock is not an important factor in the global methane budget. Its role has been considerably overestimated by most authors and organizations, such as FAO, IPCC and WWF.

The role of livestock-born methane in the global methane budget is completely dwarfed once the substantial amounts of methane that occur (as clathrates) in and below the permafrost layer in the Arctic are taken into account. It is often under pressure, having been capped by the ice layer and enters the atmosphere through cracks and mud volcanoes (pingos). Muskeg and its cousin peat also produce methane. Large amounts of methane also occur on recent sediments of continental slopes and clathrates have been found on ocean bottoms under low temperature and high pressure conditions. Such sediments are sometimes disturbed by earth movements or slumping, producing sudden gas emissions at the ocean surfaces which have been pointed to as culprits for capsized ships.

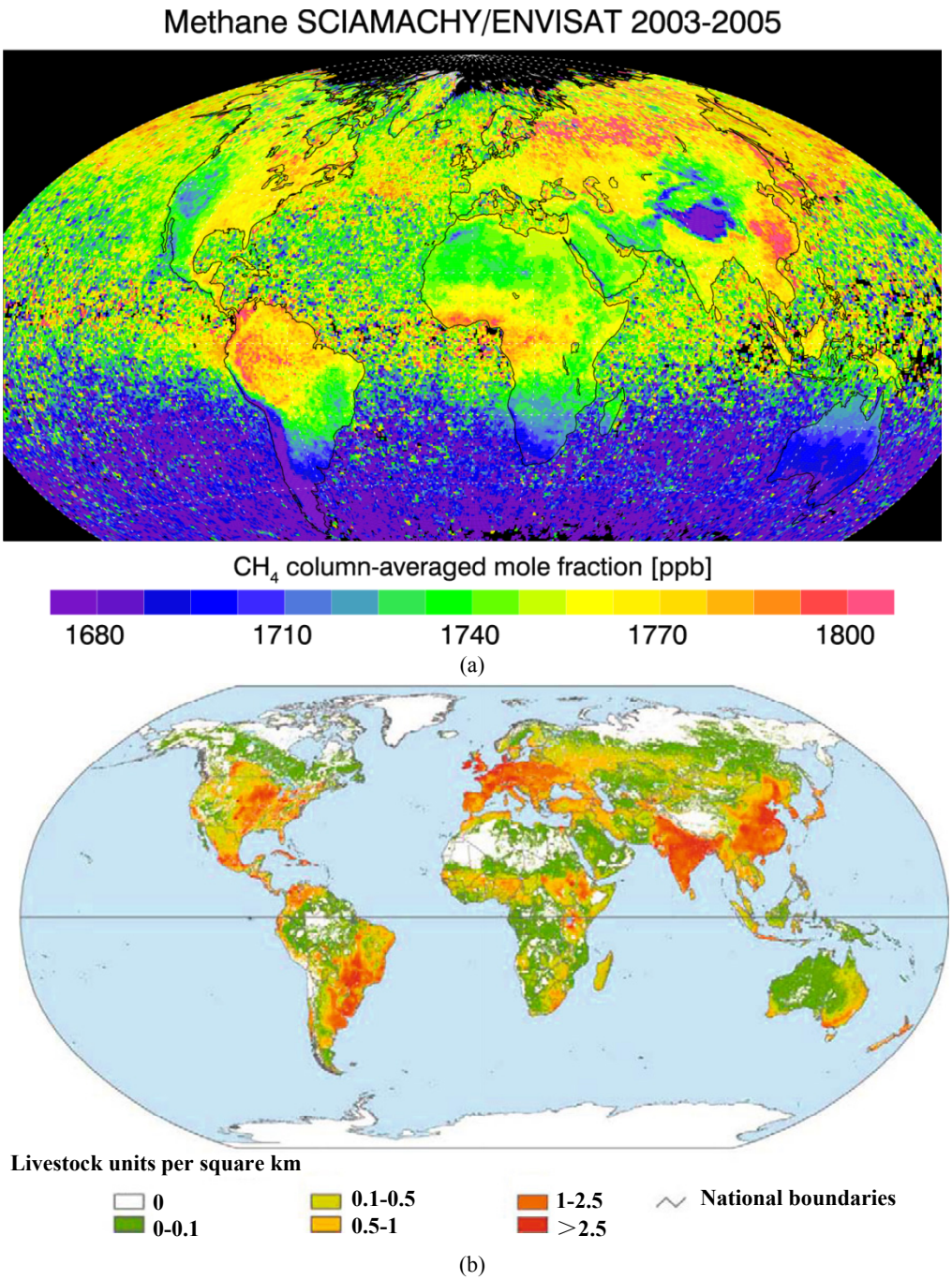

Fig. 5 (a) Global atmospheric methane distribution as measured by the ENVISAT satellite over three complete years, 2003-2005 [69, 70]; (b) Global total livestock distribution of both ruminants and monogastrics [1]. There is no discernible geographical relationship between methane and livestock distribution.

3.2.2.3 Energy Loss through Methane Emissions by Livestock

The FAO 2013 report [8] reckons that methane emissions by ruminants damages production as they constitute a waste of nutritional energy. Of course, methane emissions deliver energy to the environment, but do not spoil it, as methane is a (so far) unavoidable by-product of anaerobic degradation (by rumen cellulolytic bacteria) of the most widespread substance in the biosphere, cellulose. Without methanogenesis, hydrogen (H_2) would accumulate in the rumen and inhibit ongoing fermentation and

digestion by negative feedback [71]. Thanks to the methane emissions, ruminants can make use of the high fiber diet growing abundantly on the enormous terrestrial areas marginal to crop agriculture, and convert it into precious food for humans (meat and milk), as well as skins, fibers and other useful products. As long as there are no effective and inexpensive technologies available to manipulate rumen metabolism in order to cut back methane emissions without hampering the digestibility of fiber-rich diets, methane emissions seem to be unavoidable for the very important contribution of ruminants to food security and livelihood resources for humanity [72]. To achieve this, various enteric methane abatement strategies are being followed up in ongoing research activities, such as strengthening methanotrophic bacteria in the rumen or reducing methanogens by specific phages (bacterial viruses) while trying simultaneously to establish reductive acetogens to outcompete the methanogens for excess hydrogen in the rumen [73].

In spite of the relatively low feed-use efficiency (kg of dry matter consumed per kg of product) of ruminants grazing on the vast areas covered by grasslands at a global scale, they make use, as efficiently as possible, of the huge amounts of the otherwise useless fiber growing there in abundance. In the absence of alternative uses of huge areas it does not make sense to blame grassland based systems for low feed-use efficiency and high emission intensities, as is frequently done in modern scientific literature [5, 7, 8].

3.2.3 Nitrous Oxide Emissions and Cycling

N_2O (Nitrous oxide) is a natural atmospheric trace constituent of less than 0.000035% (vol.). It is the product of a lateral circuit of the nitrogen cycle. Whenever aerobic nitrification (bacteria mediated oxidation of ammonia to nitrate) and particularly anaerobic de-nitrification (bacteria mediated reduction of nitrate to elementary Nitrogen—N_2) takes place in the soil, N_2O is released as a leaking by-product. Both, the nitrogen quantity in circulation and the mean nitrogen turnover rate determine the nitrification and de-nitrification rates and therefore determine the quantity of N_2O released into the atmosphere (besides, of course, the prevailing site characteristics such as waterlogging or temperature or availability of easily degradable carbon sources). The dominant breakdown mechanism of nitrous oxide (to N_2 and O_2) is modulated by ultraviolet solar radiation in the stratosphere. Therefore N_2O, just like all the other GHGs, important in livestock production systems, is a natural substance, which forms part of a natural cycle.

Enteric fermentation normally constitutes an additional source of methane emissions when ruminant livestock is introduced into a pristine ecosystem, unless other sources of methane were displaced (such as wild ungulates) or natural methane emissions were reduced through management interventions such as artificial drainage.

In the case of nitrous oxide, however, the situation is not so clear cut: Grazing animals indeed accelerate nitrogen cycling somewhat; however, they do not increase the amount of nitrogen in circulation. Therefore, nitrous oxide emitted from manure is by no means additionally released by livestock. Herbage and other plant biomass also produce considerable amounts of nitrous oxide (N is mineralized, nitrified and de-nitrified) even without passage through livestock intestines. It could well be that N_2O emission rates from native forests (with often high N content in the leaves) are even greater than from managed grasslands. In this case the 23 kg of CO_2 equivalent per kg carcass weight (emitted as N_2O) charged to the beef industry in South America by Gerber et al. [8], which should be reduced to zero or even adopt a negative value, when the grassland is situated at a formerly forested area. This applies even more to grasslands sown with certain species such as *Brachiara* spp. which exert BNI (biological nitrification inhibition) in the rhizosphere, hence considerably reducing N_2O emissions [74]. In any case, this number has to be corrected by the amount of

N_2O that would be released by the pristine ecosystem anyway, even if it had not been altered by management or even if the biomass had not passed through the animal's stomach. On the other hand, the application of nitrogen fertilizer (which is rarely done on extensive grazing land because of economic constraints) certainly increases the chances of N_2O emissions by the elevated quantity of nitrogen in circulation. Nitrogen fertilization is practiced, however, to a far higher degree in (forage) cropping than in true pastoral systems.

The IPCC [60] in its "Guidelines for National Greenhouse Gas Inventories" meticulously provides N_2O emission factors for all the potential sources of nitrous oxide emissions from managed ecosystems, such as total Nitrogen deposited (as fertilizer, cured manure or fresh dung and urine) or mineralized from crop residues or soil organic matter. All the various N_2O sources in managed ecosystems are taken into account by the IPCC, however, no corrections are carried out for emissions from natural baseline scenarios, which would occur anyway in the pristine ecosystems (replaced by the respective agro-ecosystems), even without human intervention. Therefore, net anthropogenic N_2O emissions from managed ecosystems are systematically overvalued.

4. Conclusions

Just like CO_2, non CO_2 GHGs, methane and nitrous oxide, are also part of natural cycles. Rather than considering the actual emissions, one ought to take into account the observed or theoretical difference of atmospheric steady state equilibrium concentrations (between sources and sinks) before and after the creation of a new or additional source of emission which on the other hand might also alter the sink intensity through substrate induced enhanced breakdown rates (auto-catalytic response). If at all, only this difference in the steady state equilibrium concentration of a GHG in the atmosphere could exert any influence on the climate.

CO_2, CH_4 and N_2O concentrations have been increasing in the atmosphere in the past decades [65] most likely due to human activities. However, this trend does not seem to be driven by livestock-born emissions. This can be shown in the case of methane, where no livestock signal is discernible in historical emission rates or in global methane distribution (Sections 3.2.2.1 and 3.2.2.2).

As far as carbon dioxide is concerned, animal husbandry per se is CO_2 neutral (as livestock-born CO_2 emissions by respiration are offset by photosynthesis of herbage regrowth), except for emissions from fossil fuels burned during the production and marketing process, and the one-time emission (or sequestration) from land use change. Fossil fuel consumption is particularly high in intensive factory farming systems with cultivated herbage and grain, transported and fed to animals in confinement, whereas in extensive grazing systems fossil fuel consumption (and the associated CO_2 emission) is very low and can even be zero. For that reason, Ruviaro et al. [75] found the smallest carbon footprint from beef production in grazing systems with fairly high quality forage and therefore, short fattening periods.

Well managed grasslands are stable ecosystems with no net CO_2 emissions and with considerable C storage capacity. Normally, the one-time emission from deforestation for pasture establishment becomes negligible per unit of product once spread out over the accumulated production for the entire period of pastureland utilization (which easily can be hundreds of years). Moreover these emissions have to be corrected by the carbon storage capacity of subclimax grasslands. However, these requirements remain usually disregarded in the scientific practice of emission intensity and life-cycle assessments (emission per kg of product).

As far as non CO_2 GHGs are concerned, baseline emission scenarios over time and space are not taken into account (as shown in sections 3.2.2 and 3.2.3) by

(almost?) all the authors of publications on "life-cycle assessments" [37], nor by the IPCC [60] in its "Guidelines for National Greenhouse Gas Inventories" (which most authors refer to). Even the most recent assessment of carbon footprints in different beef production systems in South America [75] does not consider pristine ecosystem or pre-climate change baseline scenarios of GHG-emissions. It is obvious though that the emissions from managed ecosystems need to be corrected by these baseline emissions in order to determine the true anthropogenic part of any "carbon footprint". Therefore, only part of the emissions of non CO_2 GHGs from managed ecosystems can be considered as human induced, i.e. as far as they exceed the natural emissions from the respective pristine ecosystems (now replaced by agro-ecosystems) or from the respective pre-climate-change scenarios. This principle is also recognized by the lead author of the above mentioned paper [75]. It has, however, been overlooked by the IPCC [60] which has been serving as the leading reference for carbon footprint, emission intensity, and life-cycle assessments, hence leading to considerable overestimations of emissions from livestock and cropping. This important methodological deficiency is consistently propagated through recent scientific literature.

Considering all these factors, grass-fed beef should be highly competitive with pork, poultry and any other kind of meat as far as potential climate impact is concerned, even if there were detectable climate sensitivity to anthropogenic greenhouse gas emissions, which is far from certain. The straightforward conclusion from the discussion above is that domestic livestock's and particularly grazing animals' contribution to climate change to any noticeable extent is very unlikely.

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The Effect of Herb Feeding on Antioxidant Liver Activity

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Abstract: The aim of the study was to evaluate the effect of 1.5% herb supplement of RL (rosemary leaves), YB (yarrow blooms), PL (plantain leaves), OS (oreganos talks) or red GP (grape pomace) on the broiler liver antioxidant activity. Samples were analyzed by FRAP (ferric-reducing antioxidant power), FRK (free radicals) and DPPH (2,2-diphenyl-1-picrylhydrazyl) methods. Oxidative stress values (metallo thionein, reduced glutathione, oxidized glutathione and reduced/oxidized glutation ration) were measured in the blood and liver. Biochemical parameters (serum albumin, uric acid and bilirubin) were monitored in the blood. The antioxidant activity measured by the FRK method was higher in the oregano supplement ($P < 0.05$) than in plantain and rosemary supplements.

Key words: FRAP, FRK, DPPH, antioxidant activity, herb.

1. Introduction

Earlier breeders were only focused on increasing yields. Nowadays, it is known that good health is an assumption of good animal performance. Moreover, consumers are not only interested in price, nutrition value, origin, taste and quality of purchased animal products. They also start to take care about living conditions in which animals are kept. Safe and optimal animal handling is required in conventional poultry breedings as well as in other livestock breedings. Furthermore, consumers are afraid of using antibiotics, hormones etc. in livestock production.

European Union has banned using of antibiotics like growth stimulators in the diet for all member states since January 2006. Therefore, it is an effort to find out herbs with a positive effect on animal health. The research was conducted to detect the effect of herb feeding on the antioxidant activity and selecte biochemical markers and antioxidants in chickens.

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2. Materials and Methods

The experiment was carried out on 192 female chickens during 35 days. One day old hybrid Ross 308 were used. The average weight of chickens was 43.8 g. Chickens were kept in double-deck cage technology and in the top part of double-deck cage technology during the first 10 days. All chickens were fed by the complete feed mixture BR1 (Broiler No. 1) for the first 10 days (Table 1). Thereafter, female chickens were divided into 6 groups (Table 2). Each group had 3 repetitions with 10-11 members. Chickens were fed by the complete feed mixture BR2 after the first 10 days. Chickens were fed ad libitum. The diet was given twice daily. In the last week of the experiment, the diet was supplied three times weekly. The compositions of complete feed mixtures are shown in Table 1. Main components of complete feed mixture BR2 were the same for all chickens in all groups. The experimental group was fed by the complete feed mixture BR2 with 1.5% herb supplements. Herb supplements were RL (rosemary leaves), YB (yarrow blooms), PL (plantain leaves), OS (oregano stalks) or

red GP (grape pomace). The CO (control group) was fed by the complete feed mixture BR2 with 1.5% wheat supplement (Table 2).

The temperature, humidity, light intensity and air convection were monitored during the experiment. The temperature was 30 °C in the shed in the first day. Thereafter, the temperature reduced gradually to 20 °C. It was difficult to maintain required indoor temperature because of high outside temperature. The relative humidity was around 60%. The light intensity and light mode were regulated (Table 3). The light intensity was 40 lux for the first 15 days of the experiment and 20 lux from 15 to 35 days.

Six chickens from every group were killed by decapitation the 35th day of the experiment. The average weight of live chickens was 1,770 g. Samples of blood and liver were collected immediately after the decapitation. Livers were stored in polystyrene box with the ice. Liver samples were processed during the day of decapitation.

The antioxidant activity in the blood was measured by FRAP (ferric-reducing antioxidant power), FRK (free radicals) and DPPH (2,2-diphenyl-1-picrylhydrazyl) methods. MT (Metallothionein), reduced GSH (glutathione), oxidized GSSG (glutathione), GSH/GSSG ratio as oxidative stress values and albumin, uric acid, bilirubin as biochemical parameters were measured in the blood. MT, GSH, GSSG and GSH/GSSG values were measured in blood and liver. The antioxidant activity was expressed by a TE (trolox equivalent).

Spectrophotometric measurements of antioxidant activity were carried out using an automated chemical analyser BS-400 (Mindray, China). The determination of antioxidant activity was carried out by FRAP, FRK and DPPH methods as described by Sochor et al. [1]. The absorbance of samples in the FRAP method was measured at 605 nm for 10 min. The absorbance of samples in the FRK method were measured at 450 nm for 10 min. The absorbance of samples in the DPPH

Table 1 The composition of complete feed mixture BR1 and BR2.

Components	BR1 (%)	BR2 (%)
Wheat	30.0	41.5
Corn	30.0	22.0
Soybean meal	32.0	27.0
Rape-oil	4.0	4.0
Herbs*	0.0	1.5
Premix	4.0	4.0

*Rosemary, yarrow, plantain, oregano or red grape pomace.

Table 2 The scheme of the experiment.

Supplements	Herb portion (%)	Number of repetitions	Number of chickens
RL	1.5	3	30
YB	1.5	3	30
PL	1.5	3	31
OS	1.5	3	30
GP	1.5	3	31
Wheat	1.5	3	31

Table 3 The light schedule in the chicken shed.

Days of the experiment	The length of lighting (h)
1-7	23
8-33	18
34-36	23

method were measured at 505 nm for 10 min.

The statistical analysis was performed using program UNISTAT 5.1 (UNISTAT Ltd., England). The blood and liver characteristics were expressed as the mean. Data variability was quantified by the coefficient of variation. Differences between groups were analyzed by Kruskal-Wallis one-way analysis of variance.

3. Results and Discussion

Synthetic antioxidants are highly effective and permanent. However, they can have an undesirable effect on enzymes in human body. Therefore, their usage is regulated in many countries around the world. That is why there are efforts to find out new and safety antioxidants from nature sources. Nowadays, it is greater attention focused on natural antioxidants. It is expected that nature antioxidants do not protect only against lipid peroxidation, but they also protect body cells against oxidation [2]. Many bioactive components contained in herbs and spices are tested on their anticarcinogenic properties in animals. Several metabolic disorders and few age-related degenerative disorders are closely associated with oxidation processes in the body. Nevertheless, the usage of herbs like antioxidant sources requires further investigations. Current studies are focused on the verification of the antioxidant capacity of herb and spices immediately after harvest as well as testing their effects on oxidative indicators. Herb supplements are important not only for chicken nutrition, but also for human health. Currently, it is recommended the consumption of herbs and spices that are rich in bioactive components [3].

The aim of the experiment was to evaluate the effect of 1.5% herb supplement in the chicken diet. The attention was focused on the oxidative stress that is well detectable in the blood and liver. FRAP, FRK and DPPH methods were used. Oxidative stress values were MT, GSH, GSSG, GSH/GSSG ratio in the blood and liver. Biochemical parameters (albumin, uric acid and bilirubin) were monitored in the blood.

Our results showed some differences among herbs in antioxidant activity in the chicken blood. The effect of 1.5% herb supplements in the diet on the antioxidant activity is shown in Table 4. The FRAP method showed the highest antioxidant activity ($P < 0.05$) in the blood in CO group (4.2 μM TE). On the other hand, Shahidi [2] found the highest antioxidant activity for the rosemary leaf extract. The lowest antioxidant activity detected by FRAP method had PL group (2.0 μM TE). The difference 2.2 μM TE was statistically significant ($P < 0.05$). Moreover, difference 1.9 μM TE was statistically significant ($P < 0.05$) between GP group (3.9 μM TE) and PL group. FRAP method shows only ability of substances to reduce ion Fe^{3+}. Therefore, it may not be positively correlated with overall antioxidant activity of the sample [4].

The highest antioxidant activity in blood ($P < 0.05$) was reached for 1.5% supplement of OS (3.9 μM TE) and for CO group (3.9 μM TE) measured by FRK method. Differences were statistically significant ($P < 0.05$) between OS group, CO group and GP group (3.8 μM TE) on the one hand and RL group (3.5 μM TE) and PL group (3.5 μM TE) on the other hand.

The highest antioxidant activity in the blood (1.6 μM TE) measured by the DPPH method was found in

Table 4 Mean values of antioxidant activity in the blood of broilers fed with the supplement of CO RL, YB, PL, OS or red GP.

Methods	CO	RL	YB	PL	OS	GP
FRAP	4.2a	2.7b	3.8a	2.0b	2.6b	3.9a
FRK	3.9b	3.5a	3.7ab	3.5a	3.9b	3.8b
DPPH	1.1b	0.8b	1.6c	0.8b	0.6ab	0.2a

a, b, c—different letters mean differences at $P < 0.05$.

the group with 1.5% supplement of YB ($P < 0.05$). On the other hand, the lowest antioxidant activity measured by the DPPH method was observed in the GP group (0.5 μM TE) in comparison with CO group (1.1 μM TE), RL group (0.8 μM TE), YB group (1.6 μM TE) and PL group (0.8 μM TE). The difference was statistically significant ($P < 0.05$). Jang et al. [5] also confirmed the positive effect of herb supplements on antioxidant activity measured by DPPH method. DPPH method is one of the basic techniques for the assessment of antiradical activity insubstances [6].

The expression and induction of MT is related with oxidative stress and cells apoptosis [7]. The MT concentration (Table 5) was higher ($P < 0.05$) for PL (2.1 μM TE) than for RL (1.9 μM TE) and CO (1.9 μM TE) in the blood. No differences was found between other groups. There was no statistically significant effect on the MT concentration in the liver (Table 6). The highest level of MT was measured for YB group (11.8 μM TE) and the lowest for PL group (8.1 μM TE) in the liver. MT is part of antioxidant defense system of liver cells [8].

GSH protects the brain against oxidative stress. It acts like antioxidant and also prevents against lipid oxidation [8]. Level of GSH (Table 5) was lower ($P < 0.05$) for YB group (6.6 μM TE) then for OS group (10.6 μM TE) and CO group (11.2 μM TE) in the blood. In the liver, the greatest difference of GSH levels was detected between OS group (24.2 μM TE) and GP group (16.2 μM TE). The difference 8.0 μM TE was statistically significant ($P < 0.05$). Livers are more sensitive to chemical harm if the level of GSH is reduced. If the level of GSH is common in the liver, chemical compounds are conjugated with GSH [9].

The lowest level ($P < 0.05$) of GSSG (Table 5) was found for RL group (1.5 μM TE) in the blood. The highest level ($P < 0.05$) of GSSG had CO group (2.4 μM TE) in the blood. In the liver, the differences of GSSG levels between GP group (17.8 μM TE), CO group (15.4 μM TE) and RL group (5.9 μM TE) were statistically significant ($P < 0.05$). OS group (10.6 μM TE) had higher level ($P < 0.05$) of GSSG in comparison with CO group and RL group in the liver. The highest level of GSSG had GP group (17.8 μM TE) and the lowest level had RL group (5.9 μM TE) in the liver.

There was no statistically significant effect on the GSH/GSSG ratio (Table 5) in the blood. However, the ratio of GSH/GSSG was higher ($P < 0.05$) for RL group (5.7 μM TE) than for GP group (1.1 μM TE) in the liver. The ratio of GSH/GSSG is an indicator of cellular health [8].When an organism is in oxidative stress, GSSG grows up and GSH/GSSG ratio goes down.

Table 5 Mean values of oxidative stress in the blood of broilers fed with the supplement of CO, RL, YB, PL, OS or red GP.

Parameters (μM TE)	CO	RL	YB	PL	OS	GP
MT	1.9a	1.9a	2.0	2.1b	2.0	2.0
GSH	11.2b	9.0	6.6a	9.0	10.6bc	7.7ac
GSSG	2.4b	1.5a	1.7	1.7a	1.9	1.9
GSH/GSSG	4.9	6.3	13.5	5.2	5.6	4.2

a, b, c—different letters mean differences at $P < 0.05$.

Table 6 Mean values of oxidative stress in the liver of broilers fed with CO, RL, YB, PL, OS or red GP.

Parameters (μM TE)	CO	RL	YB	PL	OS	GP
MT	9.1	10.1	11.8	8.1	8.8	11.6
GSH	17.9a	29.4b	17.9a	25.7 b	24.2b	16.2 a
GSSG	15.4b	5.9a	11.0bc	9.2ac	10.6c	17.8b
GSH/GSSG	1.3a	5.7 b	1.7a	2.8 b	2.6 b	1.1 a

a, b, c—different letters mean differences at $P < 0.05$.

Table 7 Mean values of monitored biochemical parameters in the blood of broilers fed with CO, RL, YB, PL, OS or red GP.

Parameters	CO	RL	YB	PL	OH	GP
Albumin (g/L)	15.9	15.8	15.3	15.2	15.7	15.5
Uric acid (mmol/L)	467.6b	393.2b	245.4a	398.1b	400.6b	236.5 a
Bilirubin (mmol/L)	3.6a	4.7ab	5.1b	5.1b	5.9b	4.0a

a, b, c—different letters mean differences at $P < 0.05$.

Biochemical parameters in the blood are shown in Table 7. The 1.5% supplements of RL, YB, PL, OS or GP had no effect on albumin concentration in the blood in chickens.

The group of GP (236.5 μM TE) and group of YB (245.4 μM TE) had lower ($P < 0.05$) concentration of uric acid than CO group (467.6 μM TE) and OS group (400.6 μM TE) in the blood. The high level of uric acid in the blood was connected with high antioxidant capacity [10].

Bilirubin values were different ($P < 0.05$) between OS group (5.9 μM TE) and CO group (3.6 μM TE) in the blood. The low level of bilirubin in the blood was correlated with a risk of pathologies in organism. Slightly increased levels ensure the protection of organisms [11].

5. Conclusions

The aim of this research was to examine the effect of herb feeding on antioxidant activity measured by FRAP, FRK and DPPH methods and selected biochemical markers like MT, GSH, GSSG, GSH/GSSG and antioxidants like albumin, uric acid and bilirubin in chickens.

The highest antioxidant activity caused by herbs was found in the blood in the OS group (3.9 μM TE) by the FRK method and in the GP group (3.9 μM TE) by the FRAP method. In chickens fed with the diet with and without herb supplements, the highest level of antioxidant activity was found in the blood in the CO group (4.2 μM TE) measured by the FRAP method. The lowest antioxidant activity was found in the blood in the GP group (0.2 μM TE) measured by DPPH method.

The lowest oxidative stress in the blood expressed by MT level was observed in the CO group (1.9 μM TE) and RL group (1.9 μM TE). The highest oxidative stress in the blood expressed by MT level was found in the PL group (2.1 μM TE). The lowest oxidative stress in the liver expressed by MT level was seen in the PL group (8.1 μM TE). The highest oxidative stress in the liver expressed by MT level was found in the YB group (11.8 μM TE). These results suggest that 1.5% supplement of PL was the most effective treatment to reduce level of MT in the liver. It is desirable low level of MT in the liver, because high level of MT indicates oxidative stress. However, the decrease of MT level in the liver in chickens treated by herb supplement was not statistically significant.

The highest level of GSH in the blood was in the CO group (11.2 μM TE). The highest level of GSH in the liver was the RL group (29.4 μM TE). The highest GSH/GSSG ratio in the blood was the GP group (4.2 μM TE). The highest GSH/GSSG level in the liver was the GP group (1.1 μM TE). The supplement of RL caused the highest GSH/GSSG ratio in the liver. It is beneficial high GSH/GSSG ratio in the liver, because GSH protects against oxidative stress.

The highest level of albumin in the blood was in the CO group (15.9 μM TE). The highest level of uric acid in the blood was in the CO group (467.6 μM TE).The highest level of bilirubin in the blood was in the OS group (5.9 μM TE).

The analysis of the effect of 1.5% herb supplement (rosemary leaves, yarrow blooms, plantain leaves, oregano stalks or red grape pomace) on oxidative stress values in the blood has shown that control group without the supplement and group with the supplement of rosemary leaves caused the lowest level of MT in blood. This suggests that the diet with the supplement of rosemary leaves caused the lowest oxidative stress in chickens. GSH/GSSG ratio and

albumin level in the blood was not influenced by herb supplements.

The supplement of oregano stalks increased the level of uric acid and bilirubin in the blood. This indicates that oregano stalks seem to be the most effective herb that increases antioxidant activity in the blood.

The best effect on the monitored biochemical parameters in the blood had the supplement of rosemary leaves in the diet. Simultaneously, rosemary leaves demonstrated the best antioxidant activity in the liver. Results in the present study indicate antioxidant activity caused by herb.

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Characterizations of Banana Peel and its Efficiency for Copper Adsorption

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Abstract: This research described the chemical and physical characterizations of banana (*Musa sapientum* Linn) peel for adsorption of copper. The FT-IR spectroscopy, BET (surface area) and SEM (scanning electron microscopy) coupled with EDX (energy dispersive X-ray) analysis were used for characterizations, while copper concentration was determined by ICP spectroscopy. The different parameters: pH values 3.0 to 9.0, banana dose (0.1 g to 0.7 g) and adsorption times (30 min to 180 min) were investigated for studying an adsorption efficient. It was found that banana peel (0.1 g) was a bio-adsorbent for copper adsorption under the suitable conditions at pH 7.0 and 90 min adsorption time. The sorption pattern was additionally found to be in linear form, according to the Freundlich and Langmuir equations with R^2 = 0.966 and 0.994, respectively.

Key words: Banana peel, copper, adsorption.

1. Introduction

Contamination of metals, especially in water is one of the majority environment problems in Thailand. The metal removals by adsorbent have been continuously interested in researches. Several adsorbents have been presented by using activated carbon [1], alumina or chaitosan [2] or zeolites [3] or chitosan [4]. The eco-friendly adsorbents have been later explored such as longan peel [5], pummel peel [6], sunflower seed husk [7] and tea or coffee residues [8, 9] because they were inexpensive and effective. These bio-adsorbents could remove metal ions due to they are mostly contained acid groups. The -OH (hydroxy) and -COOH (carboxylic) groups are mainly response for adsorption. A banana, a bio-adsorbent is also in this choice. The researches of banana used have been reported, for example, Mn [10], Cd [11], Cr(III) [12], Cr(VI) [13] and Cu [14]. Thai banana known as "Kluai Nam Wa" and scientific called "*Musa sapientum* L." is one of the most popular fruits in Thailand. The benefits of banana are not only edible that can eat in fresh form or produce as dessert or snack, but it was also interested to use as an eco-friendly adsorbent for research in removal of metals. We were then interested to applied it to use as an adsorbent for metal adsorption.

In the present work, banana peel was characterized to present the physical and chemical properties of banana peel. The efficiency of banana peel for copper adsorption was then described in term of the Freundlich and Langmuir isotherms.

2. Materials and Methods

2.1 Materials

Most chemicals were analytical grade from Ajax Finechem, Australia. Ammonia solution and Acetic acid were obtained from Merck, Germany. Stock standard solutions of 1,000.0 Cu(II) mg/L were prepared by dissolving a desired amount of $CuSO_4$ in de-ionized water in order to prepare the standard

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solutions of copper as 25.0-200.0 mg/L.

2.2 Methods

2.2.1 Preparation of Banana Peel

After a banana peel was collected from a local market, a banana peel was cut into small pieces (1-2 cm) and then dried in sun for 24 h. A dried banana peel was crushed and passed through 125 mesh of screening. A banana peel was washed with de-ionized water (at least 2 times) to remove external dirt. It was dried again in oven at 100 °C for 24 h and later kept in desicator for experimental uses. A functional group modification of banana peel was performed following Ref. [12] using 9.0 g of banana dose mixed with 5.4 mL of 0.1 M HCl and 633.0 mL of methanol solution and then stirred at 60 °C for 48 h for esterification of banana peel in order to study an adsorption efficiency after modification.

2.2.2 Characterization of Banana Peel

A Perkin Elmer (USA) model Optima 2100 DV ICP-OES (inductive couple plasma optical emission spectrometer) was used to determine copper concentration. A pH value was measured with model Eco Sense pH 10/Temperature Pen YSI Incorated (USA), SEM-EDX (scanning electron microscopy with energy dispersive X-ray) analysis model HITACHI S-3400N (Japan) and FT-IR (fourier transform infrared spectroscopy) model Nicolet 6700 Thermo Electron Corporation USA were used.

3. Results and Discussion

3.1 FT-IR Analysis

FT-IR spectroscopy was performed to characterize the chemical functional groups of banana peel. The IR spectrums are shown in Fig. 1, according to Ref. [12] where the carboxylic and hydroxyl group played a major role in removal of metal [11]. The IR bands of banana peel were appeared at 3,321.9, 2,918.5, 1,727.8, 1,369.7, 1,230.3 and 1,036.7 cm^{-1} corresponding to O-H stretching, C-H stretching, C=O stretching, C-O stretching and O-H bend, respectively. Then, the reduction of O-H stretching (3,349.7 cm^{-1}) C-H stretching (2,909.9 cm^{-1}) and O-H bend (1,726.5 cm^{-1}) were observed after modification while C=O band of ester was increased (1,740.5 cm^{-1}). The N-H deformation band was moreover found at 872.1 cm^{-1} after esterification.

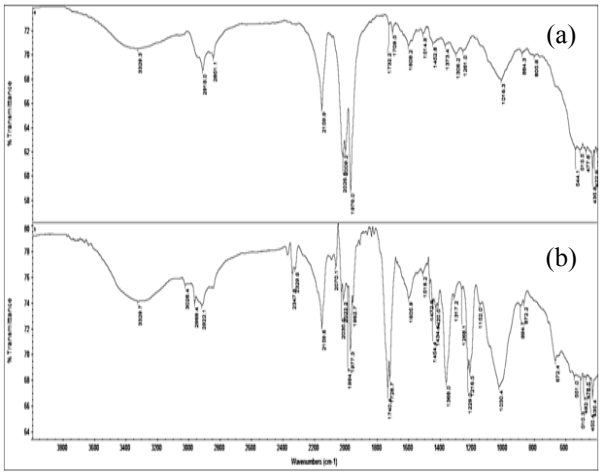
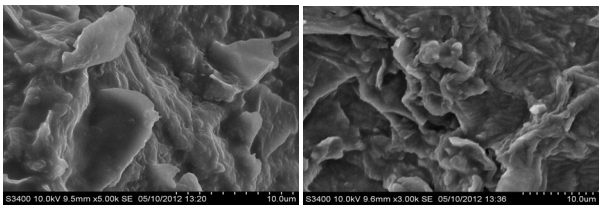


Fig. 1 FT-IR spectra of un-esterified banana peel (a) and esterified banana peel (b).

3.2 SEM-EDX Analysis

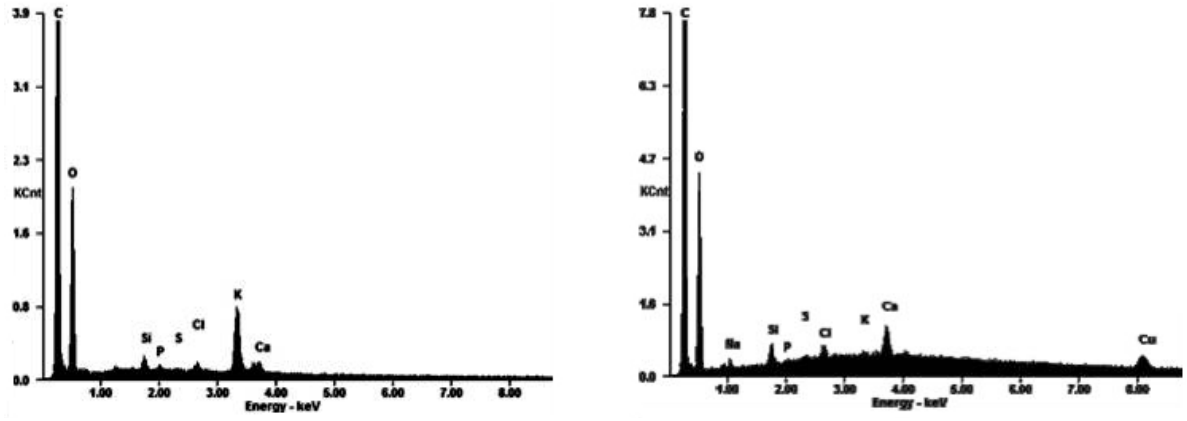
The surface morphology of banana peel was obtained using SEM. A micro porous structure was existed, shown in Fig. 2. The rough surface of banana peels, both the un-esterified and esterified banana peels, were found and revealed the adsorption of copper on banana peel surface.

Element analysis was carried out using EDX. The reports showed that a banana peel contained high amount of C and O and less amount of elements as Si, P, S, Cl, K and Ca were also obtained. After adsorption process, an additional peak of Cu was observed, shown in Fig. 3. It was confirmed that the adsorption of copper on banana peel was occurred.



(a) (b)

Fig. 2 The SEM micrograph of un-esterified (a) and (b) esterified banana peels.



(a) (b)

Fig. 3 EDX analysis of (a) un-adsorbed and (b) adsorbed banana peels with copper .

3.3 Effect of Adsorbent Doses

An effect of weight of banana peel in the range of 0.1 g to 0.7 g was investigated using 25 mg/L of copper solution and 90 min adsorption time. Increasing of banana peel weight from 0.1 g to 0.3 g was corresponded to the higher adsorption abilities from 79.2% to 94.2%. An increasing is due to more active side of banana surface to attract to copper ion. However, the percentage of adsorption was found to be constant at higher 0.3 g of banana peel because saturated active sides of banana were presented. Therefore, 0.1 g was enough for further study.

3.4 Effect of pH

A pH value was an important parameter for adsorption of metal, due to pH was concerned as the protonation of carboxylic and hydroxyl groups. An effect of pH was studied in the pH range of 3.0 to 9.0 to investigate the adsorption efficiency of banana under 90 min contact time and 0.1 g dose. This pH range was selected because of a competition between metal ion and proton on active sides of banana could be competed at lower pH (less than 4). At pH more than 4.0, the carboxylic group was deprotonated, and it became a negative charged. Hence, it increased the availability of binding sites for copper ions. From the study, the adsorption of copper was found to continuously increase with pH values from 3.0 to 8.0 and then stayed in maximum at pH 8.0 as shown in Fig. 4, where an esterified banana show higher adsorption than un-esterified one. However, at a higher pH more than 7.0, it should be carefully used because metal ions would form to hydroxide ion and be precipitated as metal oxide complexes, resulting in reductions of adsorption efficiencies of metal ions on banana surfaces. A pH 7.0 was then chosen for further study.

3.5 Effect of Adsorption Time

At the optimal pH of 7.0, the adsorption times in the range of 30-180 min were also carried out using 0.1 g of banana dose. A 25.0 mg/L copper solution was prepared to study that adsorption abilities were a function of adsorption time. The contact time seemed to be constant at longer 90 min. The percentages of

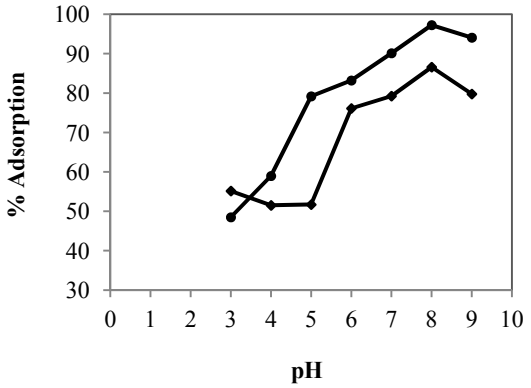


Fig. 4 The adsorption percentage of copper as a function of pH values (•) esterified and (♦) un-esterified banana peels.

adsorption from 72% to 86% were presented in this study. The longer adsorption time than 90 min was unavailable. Therefore, the contact time of 90 min was employed as the suitable time for the next study.

3.6 Sorption of Copper on Esterified Banana Peel

To study the nature of the functional groups responsibility for copper adsorption, banana peel was esterified under acid methanol. The esterification conditions were followed Ref. [12]. At an optimal pH of 7.0, the adsorption efficiency of un-esterified banana peel was less than esterified one about 11% where the adsorption value increased from 79% to 90% after esterification. Therefore, the chemical modification increases the number of acid groups, enhancing the ability of metal-binding of banana. The differences were from a modification of the functional groups which were observed from the FT-IR spectra in Fig. 1 where the N-H deformation band was confirmed at 872.1 cm^{-1} after esterification.

3.7 Sorption Isotherms

The relationships between equilibrium uptakes and the concentrations of copper were investigated to present the adsorption potentials of adsorbent using Langmuir and Freundlich equations. The Langmuir equation is shown a homogeneous adsorption, while Freundlich equations is demonstrated a heterogeneous adsorption. The equations are shown in Table 1.

The R^2 (correlation coefficient) value of the Langmuir was 0.994, representing to be good adsorption of copper on banana peel better than the Freundlich plot (R^2 = 0.966). These indicated that the homogeneous adsorption occurred on surfaces.

3.8 Regeneration of Banana Peel

In order to check the regeneration of banana peel, the desorptions of copper ion reacted banana peel were tested by eluting with acids. Due to the acids would release H^+ to replace Cu^{2+} ion in banana peel. Four acid solvents at 0.1 N of H_2SO_4, HCl, CH_3COOH and HNO_3 were tested. The regenerations (6 times), shown in Fig. 6, were observed that desorptions were not completely done. The use of H_2SO_4 seems to be better than other acids where the higher and longer recoveries with lesser variation were presented than others. However, the regeneration for 5 times could be repeated uses.

Table 1 The langmuir and freundlich parameters.

Equations	Slopes	Intercepts	R^2
Langmuir	2.229	-0.029	0.994
Freundlich	1.677	1.052	0.966

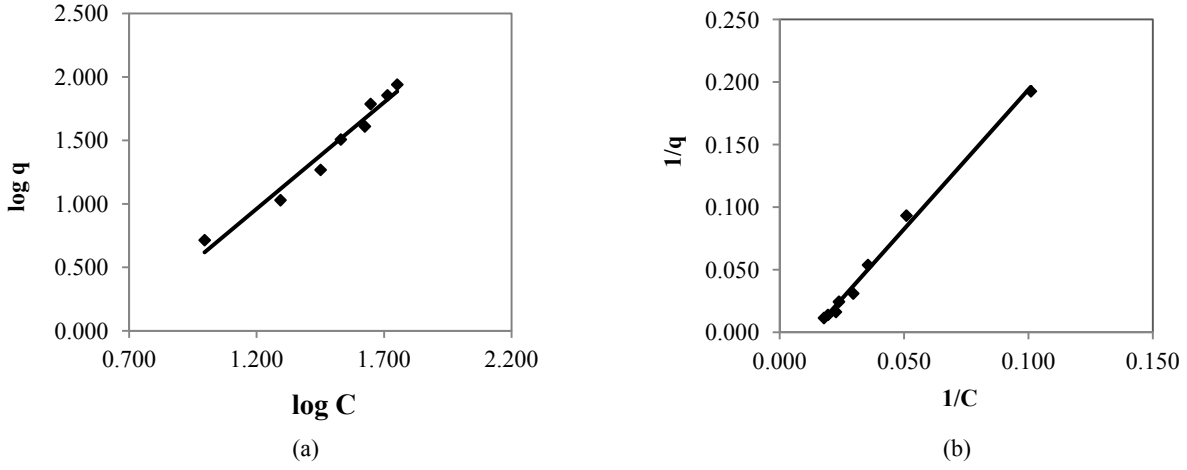


Fig. 5 The plots of adsorption isotherms of (a) Freundlich and (b) Langmuir isotherms.

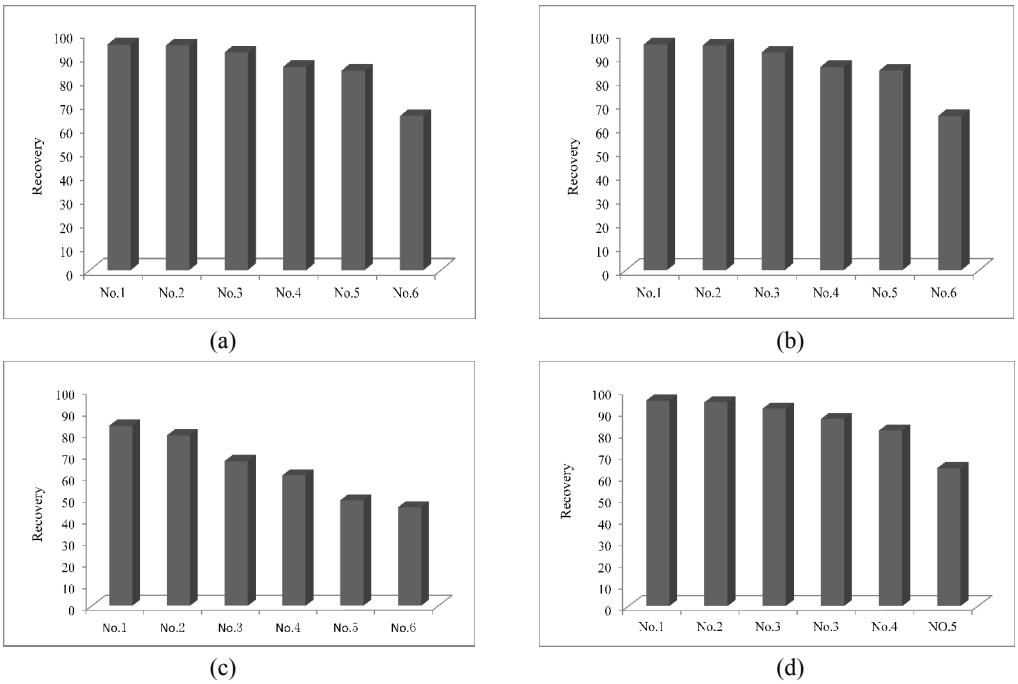


Fig. 6 Recovery (%) of Cu from banana after regeneration with acids (6 times): (a) H_2SO_4; (b) HCl; (c) CH_3COOH; (d) HNO_3.

4. Conclusions

In Thailand, Banana plant is very useful in food, feed, packing and making Thai handicrafts in many traditional ceremonies. Banana peel, a waste in this study, was used to remove metal ion. It acts as an eco-friendly adsorbent that could be used to adsorb copper ion in aqueous solutions and in water samples. Due to they contain the acid groups of hydroxyl and carboxyl groups that revealed to react with copper ions both un-esterified and esterified banana peels. However, the esterification of banana presented a better adsorption than unesterified peel because the functional groups of banana were modified. The adsorptions efficiency was depended on dose of banana peel, pH and adsorption times. The adsorption isotherm was presented using Langmuir and Frendlich equations where R^2 were satisfactory in which a homogeneous adsorption was resulted on this study. The regeneration of banana peel could be done but the use was not completely obtained.

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A Novel Approach to Ethanol Fuel Production using Rotary Collection of Forest Debris

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Abstract: In this article, the authors propose the production of ethanol from cellulose as an alternative to oil. Cellulosic-ethanol will reduce greenhouse gas emissions, and provide a means to prevent forest fires. This liquid dense fuel was selected because it: (1) easily transported and dispensed as a fuel; (2) can be handled by the existing fuel distribution infrastructure; and (3) unlike its commercial competitor, Me-OH (Methanol), Et-OH (Ethanol), is edible, thus being biodegradable and nontoxic. Forest residue ethanol is cheaper to produce and more environmentally friendly than other forms of ethanol fuel. Furthermore, forests would have less available ground fuel for fires. The potential decline of forest fires would then reduce the carbon footprint attributed directly to forest fires. In combination with ethanol fuel combustion, carbon emissions can be reduced by more than 70% compared to gasoline combustion. We used GREET (Greenhouse gases, Regulated Emissions, and Energy use in Transportation) software to assess the life cycles of different fuel pathways. In conclusion, cellulosic ethanol fuel is clearly an answer to decrease dependency on current oil imports and prevent forest fires.

Key words: Cellulosic ethanol, fuel, forest residue, forest fires, GREET software.

1. Introduction

Currently, most scientists and public officials agree on the significance of energy and its supply to ensure the maintenance of our society and its development. To date, many people around the globe are acquainted with the "nation addicted to oil" phrase used to describe the US. The domestic growing demands for oil with a decline in the domestic production capabilities have worsened the expected outcome of this so-called "addiction". Indeed, the United States Congress and President George W. Bush on August 8, 2005 enacted the Energy Bill as part of the Energy Policy Act, as an attempt to combat growing energy problems focusing on the development of new alternative sources of energy such as ethanol fuel [1]. New alternative sources that will reduce GHG (green house gas) emissions and fulfill applicable international standards of atmospheric quality are needed [2].

The 2005 Energy Bill set the goal of decreasing the importation of oil and its derivatives from the middle east by 75% by mandating a 20% reduction in the use of gasoline over the next ten years. Ethanol fuel, in the last few years, has experienced important increments in use, primarily as the oxygenated additive for gasoline after the ban of MTBE (Methyl Tertiary Butyl Ether). It has proven to be the additive of choice, as it burns cleaner with gasoline, which reduces CO_2 emissions and other byproducts [3]. However, the fact that most of the domestically produced Et-OH (Ethanol) fuel coming from corn grain (~ 90%) has led to worries that such use will cause corn prices to go up [3]. Thus, the purpose of this article is committed to explore an alternative feedstock source-cellulosic material for the production of ethanol fuel.

The US is deemed one of the largest if not the

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primary consumer of oil and its products in the world. For many years, the entire US economy has relied on the vehicle transportation sector for the delivery of goods and security assurance. The lack of stricter regulations on the average fuel economy of vehicles has caused frequent disqualifications; therefore, further compounding the transportation problems in the US [3].

The CAFE (corporate average fuel economy) regulations were first institutionalized by the US Congress in 1975 [4]. They were mainly intended to ensure improvement on the existing fuel efficiency of cars and light trucks sold in the US in the wake of the 1973 Arab Oil Embargo [4]. CAFE regulations apply to vehicles with a gross weight of 8,500 lbs or less, therefore, transportation vehicles which exceed this gross weight vehicle rating do not have to comply with CAFE standards [4].

CAFE advocates like the NHTSA (National Highway Traffic Safety Administration) and the EPA (Environmental Protection Agency) claim that most of the improvements on the fuel economy of the US vehicle fleet can be attributed to the CAFE standards themselves [4]. However, the opposition attributed most of the achieved fuel efficiency to the post Oil Embargo period in 1973—the excessive high fuel prices forced automakers to improve their products' effectiveness [5].

Most of the attacks on the CAFE standards question their efficiency at seeking better fuel efficiencies to meet the standard mileages expected of their products. It has been shown that automakers, in an attempt to meet these regulations at the lowest possible price, have opted to reduce vehicle weight which has led to weight disparities in the vehicle population [6]. Such disparities have been shown to directly correlate with increased highway deaths due to collision [6]. Indeed, a series of independent studies have demonstrated that there are about 7,700 deaths per every mile per gallon gained in fuel economy standards [6].

Fuel alternative candidates must fulfill certain criteria in order to be widely accepted. They must provide feasible solutions for the following:

(1) Economic Issues: An energy resource should be sufficient to allow for the present and future global economic development of society;

(2) Environmental Issues: An energy resource must be compatible with the long term preservation of life on earth;

(3) Strategic Issues: Present and future management of energy resources must lie in the hands of societies committed to human progress.

Et-OH meets all of the above requirements, that is, its use encompasses economic, environmental and strategic solutions from reliance on oil. Et-OH fuel can be produced from a broad number of feedstock sources, both renewable and non-renewable [7]. In addition, particular by products produced from burning Et-OH as a fuel have proven to be environmentally friendly. When in its pure state, Et-OH is considered environmentally safe, and biodegradable. Furthermore, due to the wide variety of raw feedstock from which it can be formed, it can be efficiently produced domestically [7]. To date, most of the Et-OH production comes from sugars or starches, obtained primarily from fruits and grains such as corn and wheat. Concerns were raised by market specialists as to the capability of corn growers to satisfy the demands of both industries for renewable fuel and for livestock—poultry feed and food processing.

The largest fire in US history, the Cedar Fire in 2003, killed 15 people, and burned 280,278 acres [8]. According to USGS (United States Geological Survey), wildfires are a growing natural hazard in most regions of the United States, posing a threat to life and property, particularly where native ecosystems meet developed areas [9]. Annually, billions of dollars are spent to suppress wildfires. Besides increasing erosion, landslides, and debris flows, fires can introduce invasive species and change the water quality of the region affected. USGS already provides information to identify wildfire risks and

monitors the effectiveness of treatments to reduce fuel buildup [9]. In this article, we propose the production of cellulose-based ethanol as a liberating tool from oil, reduction of greenhouse gas emissions, and as a means to prevent forest fires.

2. Methods

The US Department of Agriculture and Energy has stated that biomass sources surpass hydropower as a renewable energy source. The widespread abundance of cellulose makes it a great potential source for ethanol production. Examples of yearly renewable cellulose sources are corn stover, cereal straws, sugarcane bagasse, sawdust, paper pulp, small diameter trees and switch grass. Cellulose ethanol is extracted from its lignin-complex and fermented as corn ethanol. Lignin functions to give cellulosic biomass its structure and rigidity [10]. While cellulose and hemicellulose comprise about 70% of the chemical contents [10], lignin composes between 15% and 25% depending on the type of cellulosic biomass [11].

Ethanol produced from corn and cellulosic biomass is chemically identical. However, "well to wheel" lifecycle analysis shows that cellulosic ethanol reduces GHG emissions 80% below gasoline fuel [11]. The USDE (United States Department of Energy) and the USDA (United States Department of Agriculture) recently estimated 1.3 billion tons of cellulosic biomass is available for ethanol production. About 400 million tons of forest residues and about 1 billion tons of agriculture residues are currently available. One of the major advantages of cellulosic ethanol is that it does not affect the demand or cost of corn or other cash crops [12].

Wood is primarily composed of hollow, elongated, spindle-shaped cells that are arranged parallel to each other along the trunk of a tree [13]. Authors' group defined forest residues as those coming from tree bark, as well as falling leaves and branches. Calvin Hildebrand from Four Peaks Energy Group suggested that a circular collection of forest residue with a central processing plant would decrease transportation fuel expenditure [14]. By decreasing transportation distances, fuel consumption is decreased; therefore, a decrease in ethanol production may result.

Cellulosic biomass pretreatment requires many efficient steps for the maximization of ethanol production including high cellulose accessibility to crucial enzymes and high yields of sugar from hemicelluloses [15]. To acquire the low capital cost of ethanol, the process should be conducted under low pressure, inexpensive construction cost, low energy cost, low degradation of cellulose, and low production cost. A large number of pretreatment technologies have been studied; although, none have lowered the overall production cost [14].

GREET1.7 software has been used in this project to evaluate the energy consumption and emission released during processing of both corn and forest residue for ethanol production [16]. GREET software estimates energy use, GHG emissions (CO_2, CH_4, N_2O), and criteria pollutant emissions (volatile organic compounds, sulfur compounds, etc.) related to the fuel cycle of various vehicle and fuel combinations. It was created by the USDE, Center for Transportation Research, Argonne National laboratory, June 2001, and implemented by the EPA. GREET has been used to evaluate the energy and emissions impact associated with alternative fueled vehicles and advanced vehicle technologies in light-duty vehicles for the purpose of assessing near-and long-term transportation options. It examines more than thirty fuel-cycle pathways and analyzes the following components: feedstock (production, transportation, and storage); fuel (production, transportation and distribution, and storage) and vehicle operation (refueling, fuel combustion/conversion, evaporation, and tire/break war).

GREET allows the user the choice to input his/her own assumption or to use the GREET default assumptions. GREET default assumptions are based on figures obtained from the literature concerning

energy consumption, agricultural chemical use, and emission rates of pollutants for all pathways of fuel production and every type of vehicle. For the purpose of this analysis; a minimum change in default assumptions were made. We assumed 100% forest residue or 100% corn for ethanol production. Ethanol yield of forest residue or corn fermentation was set to be 92 gallons per dry ton. Energy use for forest residue collection was assumed to be 17,230 BTU (British Thermal Units) per dry ton as the herbaceous biomass [6].

The input spreadsheet presents basic variables for a variety of WTP (well-to-pump) and PTW (pump-to-wheels) scenarios, and identifies key parametric assumptions for GREET simulations. GREETGUI, the front-end user interface, interacts mainly with this sheet to set the parameters for the fuel pathways to be simulated in GREET. This spreadsheet acts as a bridge between the GREETGUI program and the GREET spreadsheet model running in the background, when users use the GREETGUI program to run the GREET model [16, 17].

The output consists of 27 Microsoft® Excel spreadsheets; each of which is briefly described in the GREET Operating Manual and simulates more than 100 fuel production pathways and 70 vehicle/fuel systems. GREET provides the user two kind of results; WTP Btu, or grams/mm Btu of fuel available at fuel station pump, and WTW (well-to-wheel), energy consumption as Btu/mile, and gas emission as gram/mile. Two sets of results were obtained for ethanol production pathways: one using 100% corn and the other 100% Forest residues.

3. Results and Discussion

With the national biomass potential depicted in the following diagram (Fig. 1) [18], the authors can estimate ethanol production. Even with our current forest residue, yard waste, and corn production, the nation can now meet E10 fuel standards. This use can potentially decrease up to 7% of our current oil imports. Biomass resources are mainly divided between the midwest and the west Coast (Fig. 1). The Midwest dominates ethanol fuel production with its vast nutritious croplands. By diffusing ethanol production between the midwest and west Coast, cropland may not be depleted and crop prices may not be affected.

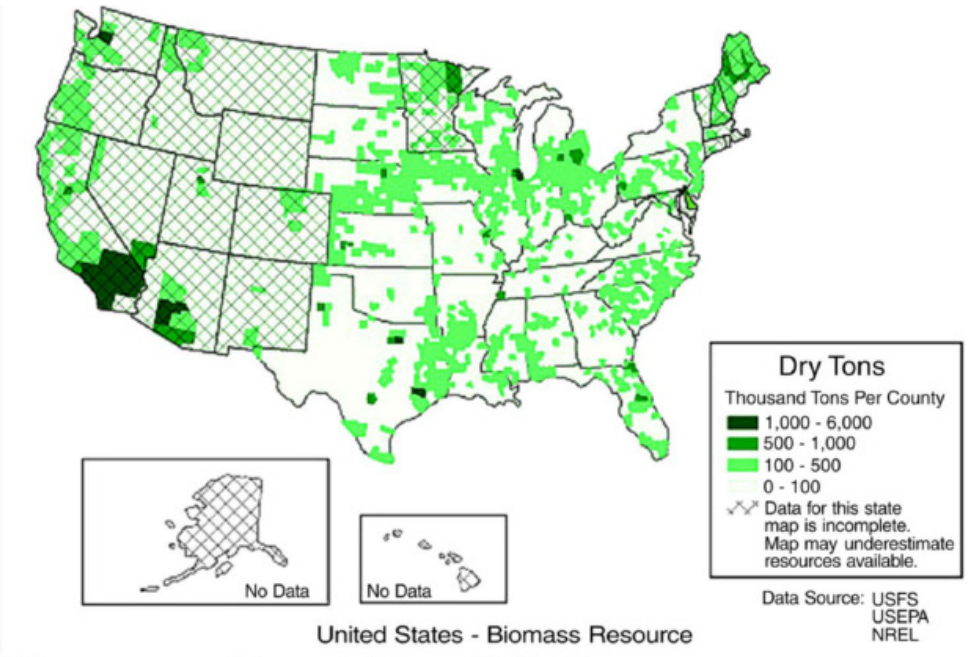


Fig. 1 Map of US biomass resource (dry tons per county).

A review of the literature demonstrates that extensive research is being conducted to find a low cost, low energy process for ethanol production [19-21]. The most common pretreatment method for the hydrolysis of cellulose into simple sugar is the use of enzymes [19]. It was shown that cellulase and pectinase enzymes were efficient in hydrolyzing cellulose, pectin, and hemicellulose into monomer sugars [19]. In that process, both orange and grapefruit peels were processed simultaneously and demonstrated the potential of mass ethanol production in Florida.

Dr. Dawson et al. [14] demonstrated that post-harvest sugar cane residue could be used to produce ethanol fuel. In that study, alkaline peroxide or acid hydrolysis was used to remove lignin (more than 50%), with acid hydrolysis proving the most effective on the removal of lignin [20]. Studies conducted by Dr. Borjesson [21] focused on the use of surfactants to increase the enzymatic conversion of softwood lignocellulose. Lignocellulose and softwood lignocellulose require large amounts of enzymes for cellulose hydrolysis. It has been shown that addition of ethylene oxide surfactants can reduce the amount of enzymes needed for ethanol fuel production. PEG (poly-ethylene glycol) was determined to increase the conversion of enzyme hydrolysis by 78% compared to the normal cellulose conversion of 48% [21]. It had been theorized that this occurs due to the number of hydrogen bonding and hydrophobic interactions between PEG and the softwood lignocellulose [21]. This binding acts as a catalyst for the enzymatic reaction.

Lastly, Dr. Zhang [20] produced plants lacking the cellulose enzyme with the hypothesis that cellulose mutations could improve the availability of cellulose for ethanol production [20]. Although successful, there are various limitations to the proposed method. Firstly, the procedure is not low cost, or low energy since the mutations are temperature sensitive. Secondly, the formulation of cellulose mutants is time consuming and not widely accepted as an alternative method. Thirdly, the relationship between soluble and insoluble substrates is not well understood. In conclusion, this would produce cellulose mutations that are not well understood, and thus, not practical.

Compared to gasoline fueled vehicles, corn and forest residue ethanol fueled cars showed a reduction in consumption of fossil fuels, CO_2 emission and GHGs potential [17]. Forest residue ethanol fueled cars produced a 30%-40% decrease in fossil fuel consumption, CO_2 emission and GHGs potential when compared to corn ethanol, fueled cars. This reduction was attributed to the fact that, more energy was used to grow, harvest and processes corn. Forest residue will consume less energy; therefore, will produce less CO_2 emission and GHGs. More reduction can be achieved, if the ethanol plants are placed in the forest within 10 miles of distance collection. The output results are shown in Figs. 2 and 3.

4. Conclusions

A solution to societal dependency on gasoline is to develop alternative fuels, such as ethanol fuel. The USDA and USDE have stated that biomass sources surpass hydropower as a renewable energy source [22]. A substantial amount of these biomass sources are from seasonal forest debris. Unmonitored forest debris along with unsafe camping practices increases the probability of forest fires, nationwide. Using forest residue as a feedstock for ethanol production in the US will save more fossil fuel and produce fewer GHG emissions than corn. Furthermore, by using forest residues for Et-OH production, forests would have less available ground fuel for fires to spread. The decline of fires would then in turn reduce the carbon footprint of forest fires. In combination with ethanol fuel combustion, carbon emission can be reduced by more than 70% compared to gasoline combustion and the growing number of forest fires. In conclusion, cellulosic ethanol fuel is clearly an answer to independence from current oil imports and ethanol production from forest residues will help prevent forest fires.

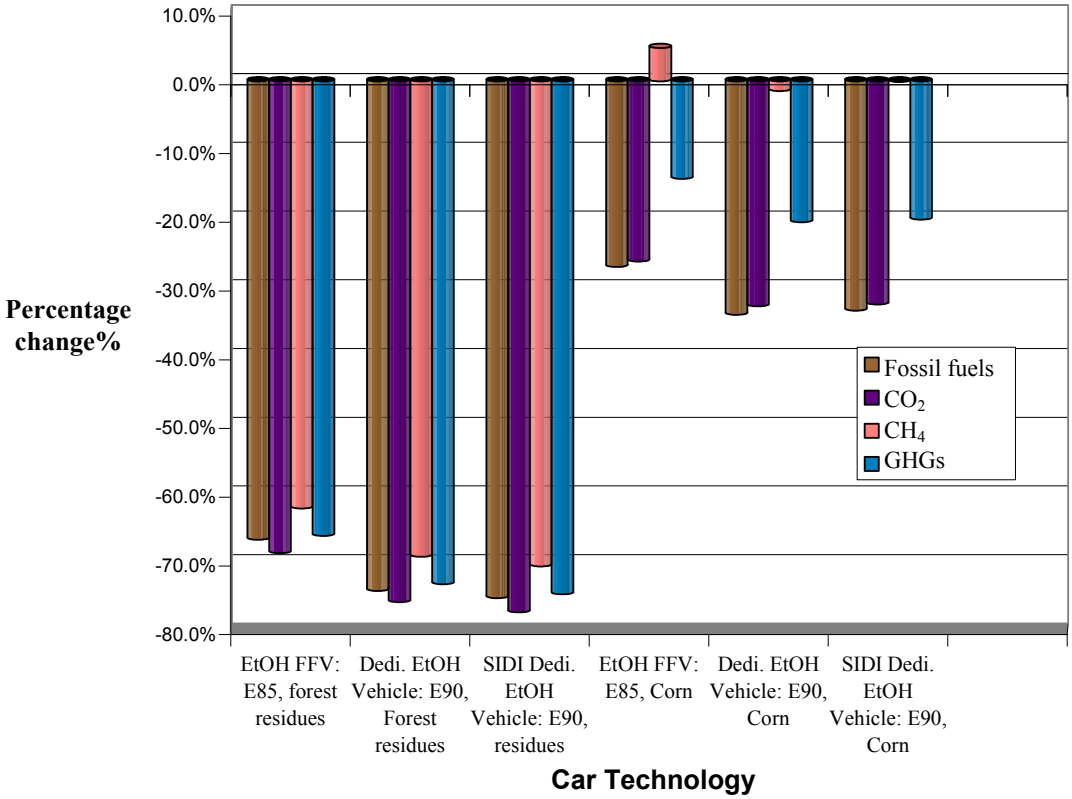
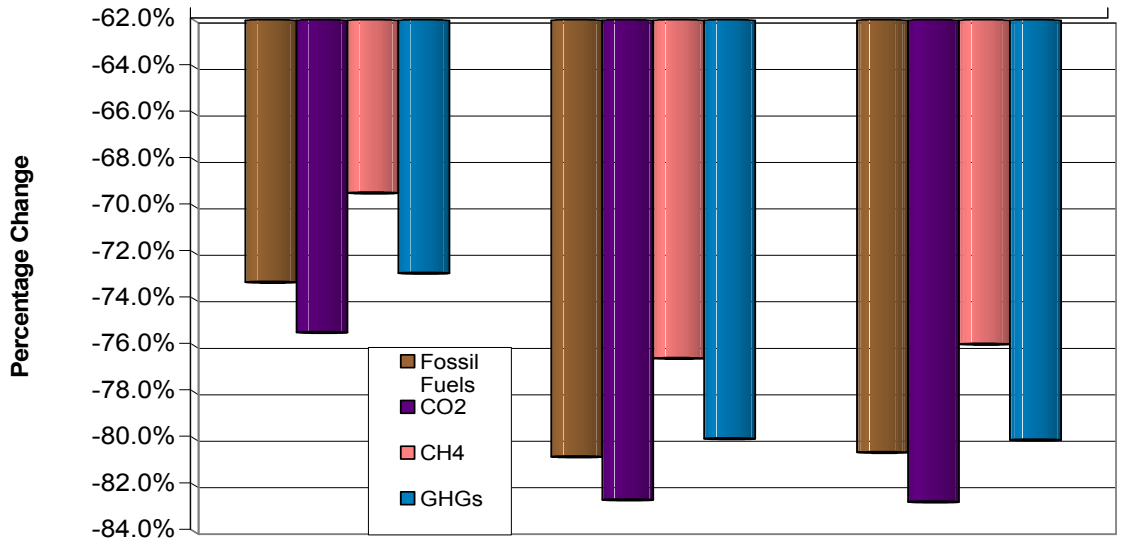


Fig. 2 Comparison of well-to-wheels energy and emission change (%, relative to gasoline fueled vehicles) for two pathways of ethanol production corn and forest residues for three different vehicle technologies.



	EtOH FFV: E85, forest residues	Dedi. EtOH Vehicle: E90, Forest residues	SIDI Dedi. EtOH Vehicle: E90, residues
Fossil Fuels	-73.3%	-80.8%	-80.6%
CO2	-75.4%	-82.6%	-82.7%
CH4	-69.4%	-76.5%	-75.9%
GHGs	-72.9%	-80.0%	-80.0%

Fig. 3 Well-to-wheels energy and emission changes (%, relative to gasoline fueled vehicles) assuming a 217,230 BTU/dry ton energy used for forest residue collection with 10 mile transportation distance to the ethanol production plant.

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