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# Cropping Systems to Improve Carbon Sequestration for Mitigation of Climate Change

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## ABSTRACT

*The recent trend of an increase in the concentration of greenhouse gases (GHGs) in the atmosphere has led to an elevated concern and urgency to adopt measures for carbon (C) sequestration to mitigate the climate change. Among all GHGs, carbon dioxide (CO<sub>2</sub>) is the most important one which occurs in the greatest concentration and has the strongest radiative forcing among all. Reducing the release of CO<sub>2</sub> to the atmosphere through “green energy” technologies or fossil fuel energy alternatives, such as wind, solar and hydraulic energies, is a major challenge. However, removal of atmospheric CO<sub>2</sub> by terrestrial ecosystems via C sequestration and converting the sequestered C into the soil organic C has provided a great opportunity for shifting GHG emission to mitigate the climate change. Soil is an ideal reservoir for storage of organic C since soil organic C has been depleted due to land misuse and inappropriate management through the long history. To optimize the efficiency of C sequestration in agriculture, cropping systems, such as crop rotation, intercropping, cover cropping, etc., play a critical role by influencing optimal yield, total increased C sequestered with biomass, and that remained in the soil. As matter of fact, soil C sequestration is a multiple purpose strategy. It restores degraded soils, enhances the land productivity, improves the diversity, protects the environment and reduces the enrichment of atmospheric CO<sub>2</sub>, hence shifts emission of GHGs and mitigates climate change.*

**Keywords:** Carbon Dioxide, Carbon Sequestration, Climate Change, Cropping System, Greenhouse Gas

## 1. Introduction

Rapid increase in carbon dioxide (CO<sub>2</sub>) concentration in the atmosphere associated with other greenhouse gases (GHGs), such as nitrous oxide (N<sub>2</sub>O) and methane (CH<sub>4</sub>), since the industrial revolution is a major concern with respect to its impact on climate change. Therefore, there is an urgency to adopt effective measures for mitigating the threat of global climate change [1]. The concentration of CO<sub>2</sub> in the atmosphere increased from 280 to 387 ppmv in 1750 to 2007, and continues to increase at the rate of 1.5 ppmv per year. During the same period, N<sub>2</sub>O was increased from 270 to 314 ppbv, and CH<sub>4</sub> increased from 700 to 1745 ppbv (**Table 1**) [2,3]. Increased concentration of GHGs impacts the temperature of the Earth by absorbing and emitting radiation within the thermal infrared range. The anthropogenic enrichment of GHGs in the atmosphere and the cumulative radiative forcing (factors affect the balance between incoming solar radiation and outgoing infrared radiation within the Earth's

atmosphere) has led to a substantial increase in global surface temperature. The major sources to enrich the atmospheric GHGs are fossil fuel combustion and land use changes. For instance, about 25% of CO<sub>2</sub>, 50% of CH<sub>4</sub> and up to 70% of N<sub>2</sub>O released globally through human sources [4]. Increased frequency of natural disasters, such as floods, tsunami, hurricane, etc., during the recent years might be attributed to the climate change associated to increased accumulation of GHGs in the atmosphere. The global surface temperature increased by 0.6°C since the late 19<sup>th</sup> century with a current average warming rate of 0.17°C per decade [2]. Such temperature increase would considerably alter the distribution of precipitation, e.g., 0.5-1% of precipitation increase per decade in the most of Northern Hemisphere and 0.3% increase in the tropics and sub-tropics [1]. Consequently, land productivity, biomass accumulation, biodiversity, and the whole environmental system would be negatively impacted. The US EPA has released its final findings on greenhouse gases and has declared that “GHGs threaten

the public health and welfare of the American people” [5]. Therefore, it is urgent to adopt practical and effective approaches to controlling the GHG emission for mitigating global climate change for a sustainable development of the environment. The objective of this review is to briefly elucidate the main sources of GHG emission and particularly address the sustainable development of cropping systems for carbon sequestration to mitigate the threat of the global climate change.

## 2. Major Sources of GHGs and their Contributions

A major source of CO<sub>2</sub> in the atmosphere is fossil fuel combustion and cement production. Total emission from the above source increased from  $5.4 \pm 0.3$  to  $7.9$  Pg C yr<sup>-1</sup> in the global scale in 1980s;  $6.3 \pm 0.4$  Pg C yr<sup>-1</sup> from the same source in the 1990s; and up to  $7.9$  Pg C yr<sup>-1</sup> from 1980 to 2005 [1,6]. Over 70% of the total emission is from combustion of liquid and solid fuels. Land use change, such as deforestation, land degradation, etc. also contribute to anthropogenic CO<sub>2</sub> emission [1,7,8], which has been constant at about  $1.7 \pm 0.8$  Pg C yr<sup>-1</sup> during 1980s through 2005. The global emission of carbon is estimated at  $270 \pm 30$  Pg due to fossil fuel combustion and  $136 \pm 55$  Pg due to land use change and soil cultivation during the last 150 years [1,9-11]. The CO<sub>2</sub> emission rate has increased dramatically since 2000, as evident from an increase from 1.1% during 1990-1999, to > 3% since 2000. This is attributed to increased energy demand with an increase in gross domestic product (GDP) [6].

Besides CO<sub>2</sub>, there are some other gases that can contribute the global climate change. However, the contribution to the greenhouse effect by different gases is determined by the characteristics of the gas and its abundance. For instance, CH<sub>4</sub> is about 8 fold stronger than CO<sub>2</sub> on a molecule-for-molecule basis, however the net contribution of CH<sub>4</sub> to the greenhouse effect is much smaller because its lower concentration than that of CO<sub>2</sub>. From the radiative forcing of the main GHGs, CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O (Table 1), it is also evident that CO<sub>2</sub> is the predominant GHG. Similarly, the other three GHGs named in the

Kyoto Protocol, hydrofluorocarbons, perfluorocarbons, and sulfur hexafluoride (US EPA, 2009) may impact the climate change but their radiative forcing is considerably low due to their very low concentrations as compared to that of CO<sub>2</sub>. Therefore, it is important to control the concentration of atmospheric CO<sub>2</sub> by reducing its emission by using fossil fuel more efficiently than ever before, and by adoption of “green-energy technologies”, such as fossil fuel alternatives, solar, wind, hydraulic energies, etc. On the other hand, terrestrial plants play a critical role to remove the atmospheric CO<sub>2</sub> via their photosynthesis and assimilation of CO<sub>2</sub> to produce plant biomass.

## 3. Carbon Sequestration for Shifting GHG Mitigation

Carbon sequestration by terrestrial vegetation, as one of the most effective options for shifting the GHG emission has been identified by the Intergovernmental Panel on Climate Change [2]. Terrestrial ecosystems associated with land use and soil management play an important role in the global C budget [1]. For example, the current terrestrial sink for carbon is estimated to hold 550-700 Pg of carbon in the world’s vegetation and 1200-1600 Pg of soil organic carbon [8]. This has shown a great potential to offset the total amount of C emitted and accumulated in the atmosphere through all possible sources.

Therefore, the United Nations (UN) Framework Convention on Climate Change (UNFCCC) has setup 3 major conventions to combat desertification, land degradation, and improving biodiversity. Furthermore, the Kyoto Protocol, negotiated in 1997, provides the framework for activities aimed at reducing emissions of GHGs. The protocol contains a joint commitment of the industrialized countries to reduce their GHG emissions by at least 5% below the levels of 1990, over the period of 2008-2012 [12].

The removal of atmospheric CO<sub>2</sub> by increasing the assimilation of CO<sub>2</sub> with terrestrial vegetation, retaining C and enhancing the transformation of atmospheric C to plant biomass and soil organic matter along with reducing GHG emission has become a worldwide strategy to

Table 1. Increase of dominant greenhouse gases and their radiative forcing [3].

Greenhouse gases	Preindustrial level	Current level	Increase		Radiative forcing (W/m <sup>2</sup> )
CO <sub>2</sub>	280 ppmv	387 ppmv	107 ppmv	38%	1.46
CH <sub>4</sub>	700 ppbv	1745 ppbv	1045 ppbv	149%	0.48
N <sub>2</sub> O	270 ppbv	314 ppbv	44 ppbv	16%	0.15
CFC-12*	0	533 pptv	533 pptv	-	0.17

\*CFC: chlorofluorocarbon

mitigate climate change. However, the efficiency of C sequestration by various vegetations and management in various systems differs greatly due to their physiological characteristics, growth rates, biomass accumulation, and environmental factors. Therefore, it is important to optimize ecosystems based on various climates and geographical characteristics to efficiently and effectively sequester C from the atmosphere and for shifting the mitigation of the climate change.

Regarding the C source and sink in the global ecosystems, there have been a lot of controversial arguments. Forest is commonly considered as a C sink because the storage of organic C accumulated in trunks and major branches of a tree can last longer and the C cycling is slower than that in annual plants. However, it is only a “time” issue because the C slowly sequestered in forests through a long term period might be easily returned to the atmosphere either through deforestation or through climate-change induced emissions. As indicated by Turner *et al.* [13], significant losses of C are associated with harvesting either for biomass energy or for wood products even for intensely managed forests. In the case of biomass energy, C is lost in one-way emission as a source through direct fuel combustion of wood. For wood products, only about 23% of merchantable wood can be harvested [14] and noncommercial parts of the tree are burned as slash or left to decompose. Also, a large fraction of the merchantable wood may become products with lifetimes of less than 5 years [13]. Wild-fires, often occurs in forest, can cause an abrupt emission of CO<sub>2</sub> to the atmosphere. In addition, prevailing afforestation projects may often not be desirable from a social point of view and it might compete for land use with food production in agriculture to meet people’s need as population grows. Afforestation may not be desirable either from the point of view in improving the biodiversity. Nevertheless, the Kyoto Protocol, in its original form focuses on forestry activities, such as afforestation and management, to improve the C sink. However, there may be a number of problems difficult to be solved for what are related to such forestry activities, particularly monitoring and verification, permanence, leakage and environmental effects in C sequestration by forest. In addition, safeguarding C stored in aboveground biomass of forests is difficult due to economic pressure that encourages logging for income returns, which definitely stimulates litter decomposition and CO<sub>2</sub> release.

## 4. Potential and Prospects of Cropping Systems for C Sequestration

### 4.1. Soil Organic C Stock for C Sequestration

There are advantages in promotion of degraded agro-eco-

systems as a potential C sink because agriculture occupies a larger portion of global land area (about 35%) than any other land use (**Table 2**). Soil organic C (SOC) in cultivated soils, where it contributes to soil fertility, might be less tempting to release through overexploitation due to slow decomposition. As matter of fact the prospects of good crop yields in the future would be jeopardized if the soil fertility cannot be maintained properly [12]. However, in agro-ecosystems that account a main proportion of the whole ecosystems, soil fertility is an important contribution to improve the biomass production and as in turn, increases the SOC accumulation by various vegetations. However, the conversion of the plant sequestered C to soil organic C, which forms recalcitrant C, plays a crucial role since the soil C can have a very stable and long residence time, hundreds and even thousands of years under most circumstances [15].

Agricultural soils under appropriate management can contain substantial amounts of soil C in the form of soil organic matter (SOM). Soils, excluding carbonated rocks, constitute the largest carbon pool, approximately 1500 Gt, which is almost three fold greater than that stored in the terrestrial biomass and twice the amount stored in the atmosphere [16]. The SOM contributes to plant available nutrients, buffers environmental stress, improve water-holding capacity, and reduce erosion. In addition, agricultural soils possess potential to restore a considerable quantity of sequestered C since a significant amount of SOC has been lost from the system due to land degradation and mismanagement. Most croplands have lost 30-40 Mg C ha<sup>-1</sup>, and most degraded soils may have lost 40-60 Mg C ha<sup>-1</sup> [17]. Restoration of such quantity of soil C via C sequestration in agro-ecosystems can, apart from removing CO<sub>2</sub> from the atmosphere, improve the sustainable production of the agriculture. In addition, compared to C passively stored in a forest, the SOC in agricultural soils can actively benefit food production and improve the agricultural sustainability. However, the historical C loss from the soil cannot be ignored since

**Table 2. Total area of land uses and their distributions worldwide (adopted from FAO, 2001).**

Land use	Area (Mha)	%
Permanent crops	132	0.9
Arable land	<b>1,369</b>	<b>9.7</b>
Permanent pasture	3,460	24.5
Forest and woodland	4,172	29.6
Agricultural land	4,961	35.2
Total land area	14,094	100.0

about 20% of the anthropogenic emissions of CO<sub>2</sub> was contributed by agriculture and land-use change [18]. Much of the historic C loss (about 66-90 Pg C) from the soil can be restored via C sequestration in 25-50 years [1] with appropriate land management. Indeed, soil has possessed a promising potential for C sequestration and C storage. A summary of soil C sequestration rates in the crop land of major countries is listed in **Table 3**, which shows that the cropland can sequester about 75-208 Tg C yr<sup>-1</sup> in US, 24 Tg C yr<sup>-1</sup> in Canada, 90-120 Tg C yr<sup>-1</sup> in the European Union, 105-198 Tg C yr<sup>-1</sup> in China and 39-49 Tg C yr<sup>-1</sup> in India [4].

## 4.2. Cropping Systems for C Sequestration

The great potential of C sequestration in cropland has provided a promising approach to reducing the atmospheric concentration of CO<sub>2</sub> for mitigating climate change. However, this approach depends on cropping systems, which may be defined as an operating system for growers to follow in their practices for crop production. An ideal cropping system for C sequestration should produce and remain the abundant quantity of biomass or organic C in the soil.

The organic C concentration in the surface soil (0-15 cm) largely depends on the total input of crop residues remaining on the surface or incorporated into the soil. It decreases soil C greatly to remove crop top from the soil by cleaning up the land [19]. Therefore, to improve C sequestration, it is critical to increase the input of plant biomass residues. Biomass accumulation can be enhanced by an increase in cultivation intensity, growing cover crops between main crop growing seasons, reducing fallow period of land, crop rotations, and intercropping systems. Biomass return to the soil can be improved by elimination of summer or winter fallow, and maintaining a dense vegetation cover on the soil surface, which can also prevent soil from erosion for SOC loss. The major strategies in developing cropping systems are discussed below.

**Table 3. Potential of soil C sequestration in cropland of major countries [4].**

Country (region)	Potential rate of C sequestration in cropland (Tg C yr <sup>-1</sup> )
U.S.A.	75-208
China	105-198
European Union	90-120
India	39-49
Canada	24

### 4.2.1. Crop Rotation

Crop rotation can improve biomass production and soil C sequestration, especially rotations with legumes and non-legumes. Growing legumes can substantially reduce the nitrogen input as chemical fertilizers, which in turn can reduce the fossil fuel consumption in manufacturing fertilizers [20,21]. Conversely, without appropriate crop rotation, soil productivity and biomass production will decrease due to an infestation increase in weeds, diseases, and insects. Increase in cropping intensity or cropping more frequently by reducing the frequency of bare land fallow in the crop rotation is another effective approach to improve biomass production and soil C sequestration. In addition, increase cropping intensity can decrease organic matter decomposition rate and mineralization/oxidation of SOC [22]. A long term (15 yrs) study with corn and soybean cropping systems indicated that the corn-soybean rotation system had the greatest productivity and returned the largest crop residues to the soil compared to monoculture of corn or soybean [23]. The above study implies that application of low carbon-to-nitrogen residues to maintain soil fertility in the major corn-soybean growing region in the U.S. would increase soil C sequestration by 13-30 Tg yr<sup>-1</sup>. This is equal to 1-2% of the estimated annual C released into the atmosphere from fossil fuel combustion in the U.S. (1.4 Pg C yr<sup>-1</sup>) [24].

### 4.2.2. Intercropping

Intercropping can improve the crop productivity due to increased efficiency of utilization of sunlight with an adequate spatial distribution of various plant architectures. Intercropping systems include row intercropping, strip intercropping, mixed cropping and relay intercropping, which depends mainly on the characteristics of various crops in spatial distribution and cropping goals. For example, row intercropping corn or sorghum with vine crops, such as climbing beans or sweet potato, can improve the productivity of the latter crop since their vines can climb on the former plants to take the advantage of space and sunlight. In which, the former plants usually may be expected to produce optimal yield because of their sacrifices to support the latter plants. For the convenience of harvests for different crops, especially applying combined harvest machines, strip cropping is preferred. Selecting crops or varieties with various maturity dates may help staggered harvest. In India, the sorghum and pigeonpea intercropping is a common practice. In this intercropping system, sorghum dominates the early stage of growth and mature in about 4 months, and the slow-growing pigeonpea flowers and ripen after the harvest of sorghum, which efficiently utilize the time and space for an optimal productivity of both



crops [25].

Intercropping can improve the crop productivity considerably. For example, based on the land equivalency ratio (LER) [ $LER = (\text{intercropping crop1/pure crop1}) + (\text{intercropping crop 2/pure crop 2})$ ], the total yields of sweet corn and southern peas with intercropping systems at different densities showed that 30-48% more land is required to produce the same yield (**Table 4**) in south Carolina [26].

There are many examples applying intercropping system to improve total crop yields and incomes. For instance, in Iowa, a strip cropping system with oats, corn and soybean on ridge-till rows showed that net returns with strip intercropping can be increased by 38% (\$ 188 vs. \$ 136 ha<sup>-1</sup>) compared with same crops in monoculture [27]. Another example is that intercropping oat, corn and soybean increased oat yield by 5%, corn yield by 12-15%, soybean yields dropped by 10% on the border rows due to the shading impact but the yield in the middle rows were much higher than that at the border to offset such a yield loss. As a result, the total soybean yield in this system was greater than that in the monoculture [28]. The total yield increase and benefit improvement from such intercropping system can be attributed to mutual benefits or synergetic effects of various crops. For instance, in the above intercropping system, the early-maturing oats efficiently utilized sunlight, soil nutrients and water to produce yields before corn fully developed to create shading and competition impacts on water and nutrients, and the corn strips can provide wind protection for oats. Soybean can fix nitrogen by rhizobium bacteria to supplement compensation of nutrient uptake by corn, and corn strips can provide an effective windbreak to protect soybean. In addition, strip intercropping can efficiently reduce the infestation by insects and pathogens of the host plants. In Yunnan province of China, the blast disease of rice was

successfully controlled by adopting a mixture of two different rice varieties instead of a typical pure stand of a single variety. This in turn decreased the need for chemical fungicides [29].

Mixed cropping is also an effective approach in the intercropping system to optimize the ecosystem for maximum plant production by planting two or more plants in a mixture. The benefits of mixed cropping are to balance the input and output of soil nutrients, suppress weeds and insects, control plant disease, resist climate extremes, such as wet, dry, hot and cold, and to increase the overall productivity with limited resources [30]. The classic example of mixed cropping is that the American “three sisters”, corn, beans and curcubits (squash and pumpkins). These plants, domesticated at different times, were together an important component of Native American agriculture. In the history, all these three plants were seeded in the same hole. The corn provides a stalk for the beans to climb on, the beans are nutrient-rich to offset what taken up by corn, and the squash or pumpkin grows low to the ground to keep weeds down and to prevent water from evaporation. With these mutual benefits, an overall optimal productivity with corresponding quantity of biomass of both underground and aboveground can be reached, which shows a potential for biomass return and soil C sequestration.

In the modern agriculture, such mixed cropping system has to be modified for the convenience of management and harvest with machinery. In addition to grazing pastures, there are a number of successful selections for the mixed cropping system in agriculture, such as wheat and chickpea; soybean and pigeon pea; peanut and sunflower; sorghum and pigeon pea; barley and chickpea; wheat and mustard; and cotton and peanut, etc. [31]. Furthermore, mixed cropping has a long history and it has been practiced in India, China and many other countries. For instance, Horrocks *et al.* [32] revealed the mixed cropping system in early New Zealand; Jahansooz *et al.* [33] reported the yield increase of wheat and chickpea in mixed cropping compared with sole cultivation in Australia; Gunes *et al.* [34] demonstrated the mutual benefits in mineral nutrients and soil moisture by mixed cropping in Europe; and Sahile *et al.* [35] showed that mixed cropping can promote proactive integrated disease management because mixed cropping of faba bean with cereals (barley and corn) can contribute to the slowing of chocolate spot epidemics and increase grain yield of faba bean in Ethiopia.

Relay intercropping, such as planting soybean into standing winter wheat between 20 and 30 days prior to wheat harvest, can efficiently take spatial and time advantages for optimal yield and eliminate the fallow period to conserve the soil and reduce water evaporation.

**Table 4. Yields of sweet corn and southern peas with intercropping at different densities [26].**

Plant density*	Corn yield (Mg ha <sup>-1</sup> )	Peas (Mg ha <sup>-1</sup> )	Land equivalency requirement (LER)
Full corn	6,272	-	-
Full peas	-	1,344	-
Low corn	4,704	896	1.41
Medium corn	5,152	896	1.48
High corn	5,600	560	1.30

\*low corn: 2,714 plants ha<sup>-1</sup>; medium corn: 3,848 plants ha<sup>-1</sup>; high corn: 4,820 plants ha<sup>-1</sup>; and peas were 12,879 plants ha<sup>-1</sup> in all intercropping plots.

The key to succeed in relay intercropping is timing and wheat row spacing for both plants to develop. If soybean is planted too early, it will become very tall and spindly due to lack of sufficient sunlight, and too late will delay the soybean development, and the spacing of 25 to 38 cm in wheat row of width is appropriate at Ohio State [36]. Too narrow wheat row spacing will limit the development of soybean plants and too wide will sacrifice the wheat yield. Since the relay intercropping can capture and utilize as much sunlight as possible, it has profound effect on the growth of intercropped soybean. Such a cropping system increases the net return and overcomes the risk of over production of one commodity and price fluctuations. Meanwhile, the land can be well covered and natural resources, especially the sunlight and soil, are efficiently utilized to produce economic yield and improve biomass accumulation.

#### 4.2.3. Cover Cropping

Growing cover crops is another effective approach to improve C sequestration and SOC storage. In the temperate region, winter cover crops, such as rye, ryegrass, oats, pea, vetch, clover, are commonly grown in fall, survived through the mild winter and grow again in spring to cover the bare lands during the off season. The biomass production of vetch and rye winter cover crops in biculture often ranges 5.7 to 8.2 Mg ha<sup>-1</sup> in the aboveground, and 372 to 880 kg ha<sup>-1</sup> belowground, which result in a total C input to the soil ranged from 6.8 to 22.8 Mg ha<sup>-1</sup> by cover crops, cotton and sorghum in rotation [37]. Sainju *et al.* [37] also reported that SOC increased by 6-8% with cover crops at 0 to 10 cm, and by 0.4% with rye in monoculture and 3% with vetch and rye in biculture at 0-30 cm. However, in the tropical or subtropical region, summer cover crops, such as sunn hemp, velvetbean, sorghum sudangrass, are prevailing species grown during the hot and humid summer to cover the bare land conserving soil and water and those summer cover crops, especially sunn hemp can produce as much as 15 Mg ha<sup>-1</sup> of aboveground biomass and 3.5 Mg ha<sup>-1</sup> belowground biomass, combined contributes to 8 Mg ha<sup>-1</sup> of organic C input into the soil within 3 months [38,39]. Therefore, cover cropping system provides an excellent strategy to improve C sequestration for mitigation of climate change.

#### 4.2.4. Companion Cropping

In organic farming for vegetable production system, companion cropping system is often practiced. For example, the use of permanent beds of companion crop grown alongside the vegetable crops (e.g., lettuce, cabbage, etc.) has been developed under various conditions, which is perceived as a possible alternative in organic crop production. Companion crops also have the poten-

tial to reduce the impact of pests and weeds to benefit the vegetable crops because of the biodiversity. However, the vegetable crop may benefit from the companion crop through a number of channels [40], for example:

**Trapping effects:** an excellent example is the use of collards to attract the diamondback moth (*Plutella xylostella*) away from cabbage because the former plant is more attractive to the pests [41].

**Biochemical pest suppression:** Some plants exude chemicals from roots or aerial parts that can suppress or repel pests and protect the neighboring plants. For instance, the African marigold (*Tagetes erecta*), which can release thiopene, a nematode repellent, making it a good companion for a number of garden crops. Allelochemicals, such as juglone found in black walnut, can suppress the growth of many plants, which can be used for weed control. The use of mown-killed grain rye as a mulch can prevent weed germination but do not affect transplanted tomatoes, broccoli, or many other vegetables.

**Nursing effects:** Tall and dense-canopied plants may protect more vulnerable species through necessary shading (e.g., ginger plant) or by providing windbreak. For instance, oats have been long used to help the establishment of alfalfa and other forages. In some cases, the nurse effect can act simply as a physical-spatial interaction function to benefit the main crop.

#### 4.2.5. Ratoon Cropping

Ratoon cropping is a technique allowing a crop to produce two or more harvests for yield from one planting. The basic requirements in ratoon cropping are that the crop has to have a well developed root system, earlier maturity and a perennial nature. Ratoon cropping has obvious advantages for crop production and soil C sequestration. For instance, ratoon cropping reduces the cost of production via savings in land preparation and planting; it has a better use of the growing season; efficiently utilize the sunlight energy; higher yields and biomass per unit area can be reached in a given period of time; less use of irrigation water and fertilizer than the main or original crop because of a shorter growth period; prevent soil and water erosion and nutrient leaching; and more productive economically compared to conventional cropping system. Ratooning sorghum [*Sorghum bicolor* (L.) Moench] or sugarcane (*Saccharum officinarum* L.) is successful [42], and the main crop should be cut at about 2.5-10 cm above the ground level after its maturity. Okra (*Abelmoschus esculentus*) is another ideal ratooning vegetable crop in tropics or subtropics, for which such ratooning can be conducted two or three times [43].

#### 4.2.6. Cropping Practices

Appropriate cropping practices, such as fertilization to adjust nutrient balance, appropriate water supply, etc.,

are important factors to optimize biomass production, improve crop growth and development. However, conventional tillage, especially the moldboard plowing, can result in rapid mineralization of SOC, which leads to SOC depletion rather than sequestration. Therefore, to enhance C sequestration in the soil, increased amount of plant residues must be returned to the soil and the soil must be kept a minimum disturbance. In addition, it is important to transfer the sequestered C into a physically or chemically stable form, such as recalcitrant C or soil organic C via slow humification or carbonization process.

The stability of organic C in plant residues or in soil pool depends largely on environmental changes, such as soil types, temperature, and moisture. However, the plant components play a major role for its organic C stability against its decomposition rate. For example, usually there are two major compartments of organic C in plants, active and inert, which might refer to labile and recalcitrant pools, respectively, in two-pool models proposed by McLaughlan and Hobbie [44]. The active organic C consists of 4 sub-components, decomposable organic C, resistant organic C, microbial biomass organic C, and humified organic C [45]. The physiological and chemical characteristics in plant residues, such as C:N ratio and lignin content, may affect the distribution of those different organic C compartments, which consequently influence the decomposition rates. There are a number of reports on C sequestration or SOC accumulation in croplands through integrated cropping systems and cropping practices, such as conservation tillage; cover cropping, crop rotation; land use restoration or shifting cultivation, and fertilization, etc. [4,16,19,37,46-49]. Obviously, soil organic C pool has a great potential to store sequestered C and integrated cropping systems associated with cropping practices has displayed the promising prospects in C sequestration from the atmosphere and shifting the mitigation of climate change.

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Unit conversion:

Mg (megagram) =  $1 \times 10^6$  g or million gram;  
 Gg (gigagram) =  $1 \times 10^9$  g or billion gram;  
 Tg (teragram) =  $1 \times 10^{12}$  g or trillion gram or million ton;  
 Pg (petagram) =  $1 \times 10^{15}$  g or billion ton; ppmv (parts per million by volume) =  $1 \times 10^{-6}$  liter;  
 ppbv (parts per billion by volume) =  $1 \times 10^{-9}$  liter;  
 pptv (parts per trillion by volume) =  $1 \times 10^{-12}$  liter.

# Evaluation of Treated Municipal Wastewater Quality for Irrigation

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## ABSTRACT

*Wastewater reuse is a useful tool in minimizing the amount of wastewater in the environment. Therefore, evaluation of the suitability of Al-Rustamiyah WWTP municipal treated wastewater for irrigation was made according to its composition and the international irrigation water quality standards. In addition, to classify water quality and to evaluate its suitability for irrigation purposes, Sodium Adsorption Ratio (SAR), Soluble Sodium Percentage (SSP) and Residual Sodium Carbonate (RSC) were calculated following standard equations and found experimentally as (2.11), (35.67) and (−12.75) respectively. Plotting the values of conductivity (EC) and sodium absorption ratio (SAR) on the US salinity diagram illustrated that most of the samples fall in the field of C3-S1, indicating high salinity and low sodium water which can be used for irrigation on almost all types of soil without danger of exchangeable sodium. Furthermore, the data indicate slight to moderate degree of restriction on the use of this treated wastewater in irrigation due to chloride hazard. RSC value is negative at all sampling sites, indicating that there is no complete precipitation of calcium and magnesium. Overall, the treated wastewater can be classified with few exceptions as suitable for irrigation use.*

**Keywords:** Wastewater Reuse, Irrigation, Sodium Adsorption Ratio (SAR), Residual Sodium Carbonate (RSC), Soluble Sodium Percentage (SSP)

## 1. Introduction

In many arid and semi-arid countries water is becoming an increasingly scarce resource and planners are forced to consider any sources of water which might be used economically and effectively to promote further development. Thus, the availability of good-quality water for irrigation is threatened in many places [1] and irrigated agriculture faces the challenge of using less water, in many cases of poorer quality, to irrigate lands that provide food for an expanding population.

The irrigation water needs can be met by using the available water more efficiently, but in many cases it will prove necessary to make increased use of municipal wastewaters [2]. The use of wastewater in agriculture has potential for both positive and negative environmental impacts [3]; with careful planning and management the use of wastewater in agriculture can be beneficial to the environment. However, the direct and indirect use of untreated wastewater in irrigated agriculture is increasing as a result of increasing global water scarcity, inadequate

and inappropriate wastewater treatment and disposal, increased food insecurity and escalating fertilizer costs [4-6]. Consequently, the reuse of wastewater for agriculture is highly encouraged [7,8] and it is a common practice for many reasons, not least of which is nutrient value and environmental protection [1,9]. Irrigation with treated municipal wastewater is considered an environmentally sound wastewater disposal practice compared to its direct disposal to the surface or ground water bodies [3,8].

Wastewater is a valuable source of plant nutrients and organic matter [10]. Nevertheless, it may contain undesirable chemical constituents and pathogens that pose negative environmental and health impacts [11]. At the same time, a number of risk factors have been identified in wastewater reuse, some of them are short term (e.g., microbial pathogens) whereas others have longer-term impacts that increase with the continued use of recycled water (e.g., salinity effects on soil). So, many guidelines have been developed to give a quality criteria and guidance on how treated wastewater (effluents) should be reused for irrigation purposes [12,13].

The amount of collected and treated wastewater is likely to increase significantly with population growth, rapid urbanization, and improvement of sanitation service coverage [14-16]. Hence, the use of treated wastewater in agriculture is one of the strategies adopted for increasing water supply in arid and semi arid countries [17,18]. Wastewater also has been used in agriculture for decades in many countries like India [15], Nepal [19], China [20], Spain [21] and Italy [22]. Under the conditions of increased freshwater scarcity at Arabian countries like Saudi-Arabia [17], Kuwait [23,24] and Jordan [25,26], the reuse of wastewater in agriculture is receiving great attention and increased recognition as a potential water source.

It is generally accepted that wastewater use in agriculture is justified on agronomic and economic grounds but care must be taken to minimize adverse health and environmental impacts. However, in Iraq such usage of treated or untreated wastewater has not been widely investigated and evaluated. In view of these facts, the present study was undertaken to characterize the secondary treated wastewater produced from Al-Rustamiyah WWTP and to evaluate its suitability for irrigation purposes as non-conventional water resources.

## 2. Materials and Methods

### 2.1. The Study Area

The Iraqi capital, Baghdad, has the highest level of sanitation provision with about 80% of the population connected to sewer conveying sewage to treatment facilities. It is located in the Mesopotamian alluvial plain between latitudes 33°14'-33°25' N and longitudes 44°31'-44°17' E. The general altitude ranges between 30.5 and 34.85 m a.s.l. Tigris River divides the city into a right (Karkh) and left (Risafa) sections (**Figure 1**). The area is characterized by arid to semi arid climate with dry hot summers and cold winters; the mean annual rainfall is about 151.8 mm [27].

The sewerage network that was established between 1960 and 1980 worked on the basis of the separate system, but a combined system has been adopted since 1980. In general, the wastewater quantities generated within the urban and rural areas of the mayorality of Baghdad are estimated at 1,426,013 and 2,354 cubic meters per day respectively. However, the capacity of all wastewater treatment plants in the mayorality of Baghdad was estimated at 789, 200 cubic meters per day, in which it represents as 55% of the total capacity of wastewater. The secondary treated wastewater effluent for Iraqi (WWTP) was designed to produce an average of final effluent quality of biological oxygen demand (BOD) and total suspended solids (TSS) as 20 and 30 mg/L, respectively to



**Figure 1.** Base map of Baghdad city.

meet the Iraqi National Standards set by the Regulation 25 of 1967. It reported that each day 500,000 cubic meters of raw sewage are discharged into Iraqi waterways [3].

In the Iraqi wastewater treatment plants, the existing pumping stations are also inefficient because of the lack of proper operation and maintenance and unavailability of spare parts. Despite this, most of the treated wastewater in the area under study (Baghdad City) was mixed with freshwater from the Diyala River and used downstream for unrestricted irrigation. Thus around 50% of the total treated wastewater generated could be reused [3].

### 2.2. Sampling and Analysis

Treated wastewater samples from Al-Rustamiyah WWTP were bimonthly collected during January 2009 to December 2009 in stopper fitted polyethylene bottles that prewashed with dilute hydrochloric acid and then rinsed several times with the effluent sample before filling them to the required capacity. These samples were stored at a temperature below 4°C prior to analysis in the laboratory. Procedures followed for analysis have been in accordance with the Standard methods for examination of water and wastewater [28]. The calibration for different chemical constituents was done by preparing low-level standard solutions using AR-grade chemicals and was periodically repeated to check the accuracy. Calcium ( $\text{Ca}^{2+}$ ) and Magnesium ( $\text{Mg}^{2+}$ ) were determined titrimetrically using standard EDTA, while Chloride ( $\text{Cl}^-$ ) was determined by standard  $\text{AgNO}_3$  titration. Carbonate ( $\text{CO}_3^{2-}$ ) and Bicarbonate ( $\text{HCO}_3^-$ ) were determined by titration with HCl. Sodium ( $\text{Na}^+$ ) and Potassium ( $\text{K}^+$ ) were measured by flame photometry and Sulphate ( $\text{SO}_4^{2-}$ ) by spectrophotometer turbidimetry. Total Suspended Solid (TSS) and Total Dissolved Solid (TDS) were de-

terminated by gravimetric method (dried at 103°C). Biological Oxygen Demand (BOD) was determined by the 5 Day BOD test while Chemical Oxygen Demand (COD) was determined in the laboratory by the standard Open Reflux Method. Other tests such as Conductivity (EC) and pH were directly measured *in situ* using portable measuring devices (HANNA instruments, HI 9811, portable pH-EC-TDS METER, Italy). Note that before each measurement, the pH meter was calibrated with reference buffer solution. Each analysis was carried out in triplicate and then the mean value was taken.

### 2.3. Indicators of Water Quality for Irrigation

Important irrigation water quality parameters include a number of specific properties of water relevant in relation to the yield and quality of crops, maintenance of soil productivity and protection of the environment. These parameters mainly consist of certain physical and chemical characteristics of water that are used in the evaluation of agricultural water quality. Numerous water quality guidelines have been developed by many researchers for using water in irrigation under different condition [29-32]. However, the classification of US Salinity Laboratory (USSL) is used most commonly. Parameters such as EC, pH, Sodium Adsorption Ratio (SAR), adjusted SAR (adj SAR) and the Exchangeable Sodium Percentage (ESP), Soluble Sodium Percentage (SSP) and Residual Sodium Carbonate (RSC) were used to assess the suitability of water for irrigation purposes. The criteria used to evaluate quality of wastewater for use in agriculture are listed in **Table 1**.

## 3. Results and Discussion

Descriptive statistics for all characteristics are presented in **Table 2**. An explanation of the observed characteristics follows in the following sections.

### 3.1. Hydrogen Ion Activity (pH)

The values of pH varied from 6.87 to 8.40 with an average value of 7.70, which indicates that the treated municipal wastewater is slightly alkaline in nature. The normal pH range for irrigation water is from 6.5 to 8.4. Irrigation water with a pH outside the normal range may cause a nutritional imbalance or may contain a toxic ion [32,33].

### 3.2. Salinity Hazard

Electrical conductivity (EC) is the most important parameter in determining the suitability of water for irrigation use and it is a good measurement of salinity hazard to crop as it reflects the TDS in wastewater. The most important negative effect on the environment caused by

agricultural wastewater is the increases in soil salinity, which if not controlled, can decrease productivity in long term [3]. EC values of experimental samples varied from 1910 to 2120  $\mu\text{S}/\text{cm}$  (mean value = 1949.78  $\mu\text{S}/\text{cm}$ ) while TDS values varied from 1164 to 1350 mg/L (mean value = 1234.6 mg/L) indicating slight to moderate degree of restriction on the use of this wastewater in irrigation due to salt build-up in soils and its adverse effects on plant growth [32]. Furthermore, the results indicted also that this type of water can be used on the soils with restricted drainage. Special salinity control management with selection of good salt tolerant plants is required. However, irrigation water with conductivity in the range of 750-2250  $\mu\text{S}/\text{cm}$  is permissible for irrigation and widely used. Satisfactory crop growth is obtained under good management and favorable drainage conditions but saline conditions will develop if leaching and drainage are inadequate [30]. It is clear that irrigation using saline water can add salt concentration to the soils and a problem may be occurred due to the increase in concentration that is harmful to the crop or landscape. Therefore, it is necessary to combine the use of wastewater with practices to control salinization, such as soil washing and appropriate soil drainage [3]. The primary effect of high EC reduces the osmotic activity of plants and thus interferes with the absorption of water and nutrients from the soil [34].

### 3.3. Sodium Hazard

Sodium content is the most troublesome of the major constituents and an important factor in irrigation water quality evaluation. Excessive sodium leads to development of an alkaline soil that can cause soil physical problems and reducing soil permeability [35]. Furthermore, irrigation water containing large amounts of sodium is of special concern due to absorbed sodium by plant roots which is transported to leaves where it can accumulate and cause injury [36]. However, there is a restriction in use of overhead sprinklers method with water contained a high level of sodium salts because these salts can be absorbed directly by plant leaves and will produce harmful effects.

The water can be used for irrigation when the concentration of sodium is about 8.0 meq/L (184.0 mg/L) [37]. Sodium concentrations in the samples varied from 123.60 to 221.0 mg/L (mean value = 171.11), indicating slight to moderate to high degree of restriction for sensitive crops on the use of this wastewater in irrigation [32]. Sensitive crops include deciduous fruits, nuts, citrus, avocados and beans, but there are many others. In the case of tree crops, sodium in the leaf tissue in excess of 0.25-0.50 percent (dry weight) is often associated with sodium toxicity [32].



**Table 1. Water quality classes for agricultural irrigation.**

Reference [30]					Reference [32]				
Salinity Hazard									
Parameters		Irrigation water classification				Degree of restriction on use			
		Excellent	Good	Permissible	Unsuitable	None	Slight to Moderate	Severe	
EC (dS/m)		< 0.25	0.25-0.75	0.75-2.25	2.25-5.0	< 0.7	0.7-3.0	> 3.0	
TDS (mg/L)		< 200	200-500	500-1500	1500-3000	< 450	450-2000	> 2000	
Soil Water Infiltration (Evaluate using EC and SAR together)									
EC (dS/m)	SAR	Degree of restriction	Remarks			Degree of restriction on use			
< 0.25	< 10	Low	Satisfactory for all crops			EC (dS/m) & SAR	None	Slight to Moderate	Severe
0.25-0.75	10-18	Medium	Satisfactory, some salt sensitive crops will be affected			If SAR 0-3 & EC	> 0.7	0.7-0.2	< 0.2
						If SAR 3-6 & EC	> 0.2	0.2-0.3	< 0.3
0.75-2.25	18-26	High	Satisfactory for most crops, salinity condition will be develop unless leaching and drainage are adequate			If SAR 6-12 & EC	> 1.9	1.9-0.5	< 0.5
						If SAR 12-20 & EC	> 2.9	2.9-1.3	< 1.3
2.25-5.0	> 26	Very high	Suitable for most salt tolerant plants, leaching and drainage are imperative			If SAR 20-40 & EC	> 5.0	5.0-2.9	< 2.9
Specific Ion Toxicity									
Degree of restriction on use					Degree of restriction on use				
	Low	Medium	High	Very high	None	Slight to Moderate	Severe		
Na <sup>+</sup> (mg/L)	-	-	-	-	< 100	> 100	> 100		
Na <sup>+</sup> (SAR)	< 10.0	10-18	18-26	> 26.0	< 3.0	3-9	> 9.0		
Na <sup>+</sup> (SSP)	< 20.0	20-40	40-80	> 80	-	-	-		
Irrigation Water Classification					Irrigation Water Classification				
Safe	Sensitive plants		Moderately to tolerant plants	Unsuitable or tolerant plants	No problem	Increasing problem	Sever problem		
Cl <sup>-</sup> (meq/L)	< 2	2-4	4-10	> 10	< 4	4-10	> 10		
Miscellaneous Effects									
Irrigation water classification					Degree of restriction on use				
	Safe	Permissible		Unsuitable	None	Slight to Moderate	Severe		
pH (pH unit)	-	-		-		Normal range = 6.5-8.4			
RSC (meq/L)	< 1.25	1.25-2.5		> 2.5	-	-	-		
HCO <sub>3</sub> (meq/L)	-	-		-	< 1.5	1.5-8.5	> 8.5		

Sodium hazard is usually expressed in terms of Sodium Adsorption Ratio (SAR) and it can be calculated from the ratio of sodium to calcium and magnesium. SAR is an important parameter for the determination of the suitability of irrigation water because it is responsible

for the sodium hazard [38], since it is more closely related to exchangeable sodium percentages in the soil than the simpler sodium percentage [39]. Sodium replacing adsorbed calcium and magnesium is a hazard as it causes damage to the soil structure. It becomes compact and im-

**Table 2. Summary statistics of the analytical data.**

Characteristics	Min.	Max.	Mean	Standard deviation
pH (standard units)	6.87	8.40	7.70	± 0.39
EC (μS/cm)	1910.0	2120.0	1949.78	± 48.75
TDS (mg/L)	1164.0	1350.0	1234.60	± 63.09
TSS (mg/L)	10.00	112.00	49.30	± 27.67
Ca <sup>2+</sup> (mg/L)	99.70	290.18	157.54	± 40.34
Mg <sup>2+</sup> (mg/L)	33.00	149.20	69.02	± 29.60
K <sup>+</sup> (mg/L)	9.00	26.90	16.38	± 5.19
Na <sup>+</sup> (mg/L)	123.60	221.00	171.11	± 22.28
HCO <sub>3</sub> <sup>-</sup> (mg/L)	24.88	73.80	45.16	± 11.07
CO <sub>3</sub> <sup>2-</sup> (mg/L)	0.00	12.30	9.80	± 3.44
Cl <sup>-</sup> (mg/L)	171.44	254.92	205.25	± 21.67
SO <sub>4</sub> <sup>2-</sup> (mg/L)	199.00	358.00	245.09	± 43.76
BOD (mg/L)	12.00	66.00	26.36	± 10.85
COD (mg/L)	36.00	80.00	53.10	± 13.90

pervious. It has been calculated as follows:

$$SAR = \frac{Na^+}{\sqrt{\frac{Ca^{2+} + Mg^{2+}}{2}}} \quad (1)$$

where: Na<sup>+</sup>, Ca<sup>2+</sup> and Mg<sup>2+</sup> are in meq/L.

For waters containing significant amounts of bicarbonate, Bower and Maasland [40] proposed a modification in the old SAR procedure to include changes in soil water composition that are expected to result due to dissolution/precipitation of lime in the soil upon irrigation. Therefore, the adjusted sodium adsorption ratio (adj SAR) is sometimes used [32], and it is an SAR value corrected to account for the removal of Ca<sup>2+</sup> and Mg<sup>2+</sup> by their precipitation with CO<sub>3</sub><sup>2-</sup> and HCO<sub>3</sub><sup>-</sup> ions in the water added [37]. It can be calculated as in reference [41] by using the following formula:

$$adj\ SAR = SAR \left[ 1 + (8.4 - pHc) \right] \quad (2)$$

where 8.4 is the approximate of a nonsodic saline soil in equilibrium with CaCO<sub>3</sub> and is substituted for the pH of water. This substitution reflects the high buffering capacity of calcareous soils. pHc is defined by:

$$pHc = (pK_2 + pK_c) + p \left( Ca^{2+} + Mg^{2+} \right) + pAlk \quad (3)$$

where p refers to the negative logarithm, K<sub>2</sub> is the second dissociation equilibrium constant of carbonic acid, K<sub>c</sub> is solubility equilibrium constant for calcite. Concentrations of Ca<sup>2+</sup>, Mg<sup>2+</sup>, CO<sub>3</sub><sup>2-</sup> and HCO<sub>3</sub><sup>-</sup> in meq/L.

The pHc can be calculated using the standard table given by reference [41] which related to the concentration values from water analysis. This concept has been found very useful for predicting the effect of sodium hazard of irrigation water on soil properties. Values of pHc above 8.4 indicate tendency to dissolve lime from soil through which the water moves; values below 8.4 indicate tendency to precipitate lime from waters applied [32].

A new adj SAR method [42] is derived which adjusts the calcium concentration of the irrigation water to the expected equilibrium value and includes the effects of carbon dioxide CO<sub>2</sub>, carbonate (HCO<sub>3</sub><sup>-</sup>) and of salinity (EC) upon the calcium originally present in the applied water but now a part of the soil water. The new adjusted SAR is termed widely as adj RNa, and the equation is as follows:

$$adj\ RNa = \frac{Na^+}{\sqrt{\frac{Ca_x^{2+} + Mg^{2+}}{2}}} \quad (4)$$

where Ca<sub>x</sub><sup>2+</sup>, a modified calcium concentration value in meq/L expected to remain in near surface soil water following irrigation with water of given HCO<sub>3</sub><sup>-</sup>/Ca<sup>2+</sup> ratio and EC available from the standard Tables [32].

The SAR value of the treated wastewater ranges from 1.43 to 3.19 (mean = 2.11), while adj SAR and adj RNa values range from 2.35 to 4.40 (mean = 3.12) and from 1.52 to 3.03 (mean = 2.03) respectively (**Table 3**). The comparison between SAR, adj SAR and adj RNa values and their standard values reflects water is suitable for irrigation.

Total salt concentration of irrigation waters should not be used as single criteria to prevent it in irrigation use.

**Table 3. Calculated irrigation quality characteristics.**

Characteristics	Min.	Max.	Mean	Standard deviation
SAR	1.43	3.19	2.11	± 0.43
adj SAR	2.35	4.40	3.12	± 0.58
adj RNa	1.52	3.03	2.03	± 0.36
SSP	21.38	50.82	35.67	± 6.75
ESP (SAR)	0.84	3.34	1.82	± 0.61
ESP (adj SAR)	2.16	4.97	3.25	± 0.80
ESP (SAR RNa)	1.00	3.12	1.70	± 0.51
pHc	7.63	8.14	7.90	± 0.3
RSC	-25.91	-7.07	-12.75	± 4.002
Mg <sup>2+</sup> Hazards	7.97	56.53	39.86	± 10.44

Even water with high salt concentration can still be used for irrigation without endangering soil productivity. High sodium content common to recycle water can cause deflocculating (breakdown) of soil clay particles, severely reducing soil aeration and water infiltration and percolation. In other words, soil permeability is reduced by irrigation with water high in sodium [35,43]. It is therefore, the best measure of a water likely effect on soil permeability is the waters SAR considered together with its EC. In this respect, the US salinity diagram (**Figure 2**) which is based on the integrated effect of EC (salinity hazard) and SAR (alkalinity hazard), has been used to assess the water suitability for irrigation [30]. When the analytical data of EC and SAR plotted on the US salinity diagram, it is illustrated that most of the treated wastewater samples fall in the class of C3-S1 indicating high salinity with low sodium water, which can be used for irrigation on almost all types of soil, with only a minimum risk of exchangeable sodium. This type of water can be suitable for plants having good salt tolerance but restricts its suitability for irrigation, especially in soils with restricted drainage [30,44]. **Figure 3** shows the positive correlation between EC and SAR with a correlation coefficient ( $R^2$ ) = 0.193. The lower values of  $R^2$  show that there is a higher variation in the EC values.

### 3.4. Soluble Sodium Percentage (SSP)

It is also used to evaluate sodium hazard. The Soluble Sodium Percentage (SSP) was calculated as in reference [29] by the following equation:

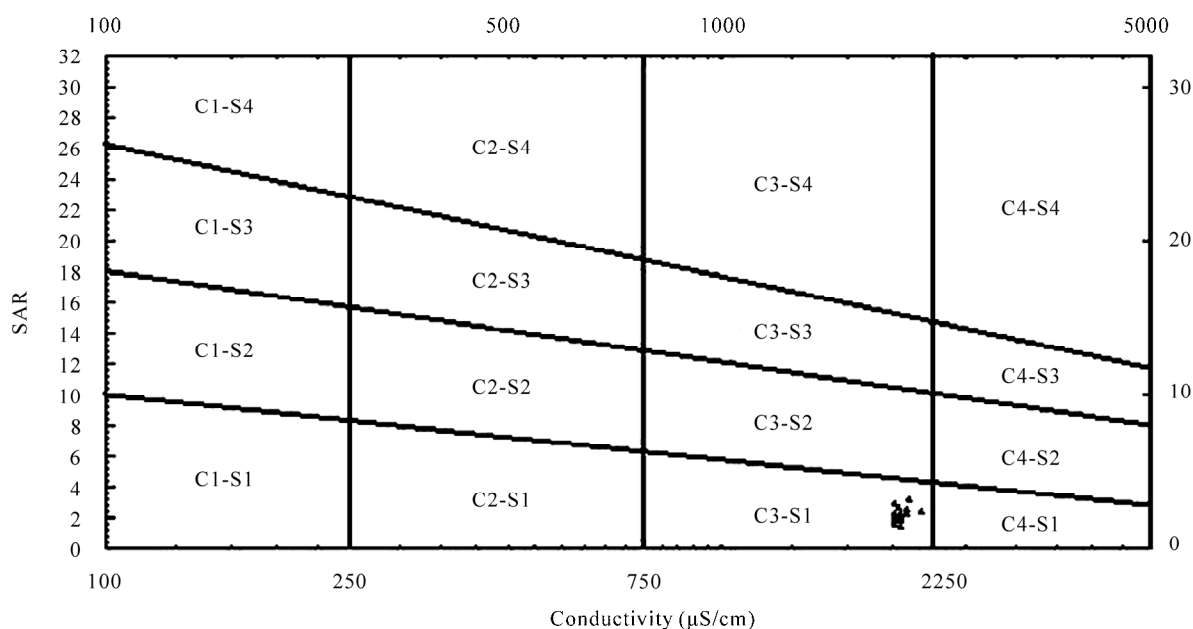
$$SSP = \frac{(Na^+) \times 100}{Ca^{2+} + Mg^{2+} + Na^+ + K^+} \quad (5)$$

where all the ions are expressed in meq/L.

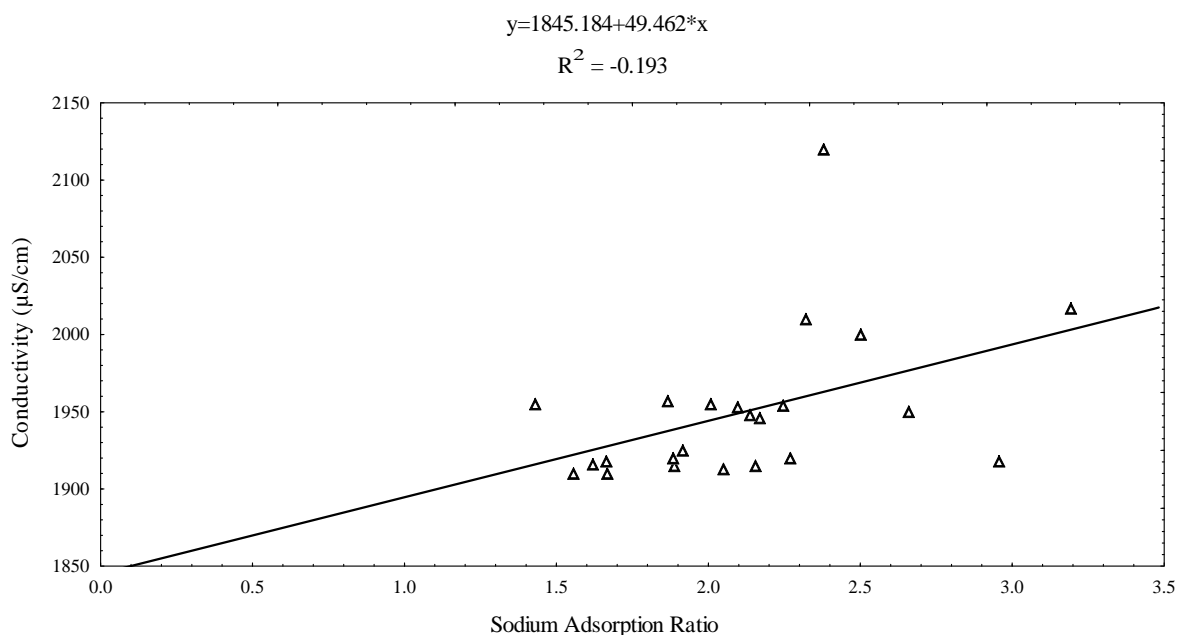
Water with SSP greater than 60 percent may result in sodium accumulations that will cause a breakdown of the soil's physical properties [45]. The calculated values of SSP varied from 21.38% to 50.82% (mean value = 35.67%) indicating moderate degree of restriction on the use of this wastewater in irrigation. When the concentration of sodium ion is high in irrigation water,  $Na^+$  ion tends to be absorbed by clay particles, displacing  $Mg^{2+}$  and  $Ca^{2+}$  ions. This exchange process in soil reduces the permeability and eventually results in soil with poor internal drainage [32]. **Figure 4** shows the positive correlation between SSP and SAR with a coefficient of 0.786.

Irrigation with waters that have high concentrations of  $Na^+$  ion relative to divalent cations may cause an accumulation of exchangeable  $Na^+$  on soil colloids. Continued uses of alkaline waters for irrigation in a closed system may have adverse effects on soil physical properties [46,47], deteriorate the soil and water resources of the region and affect the sustainability of crop production in the long run.

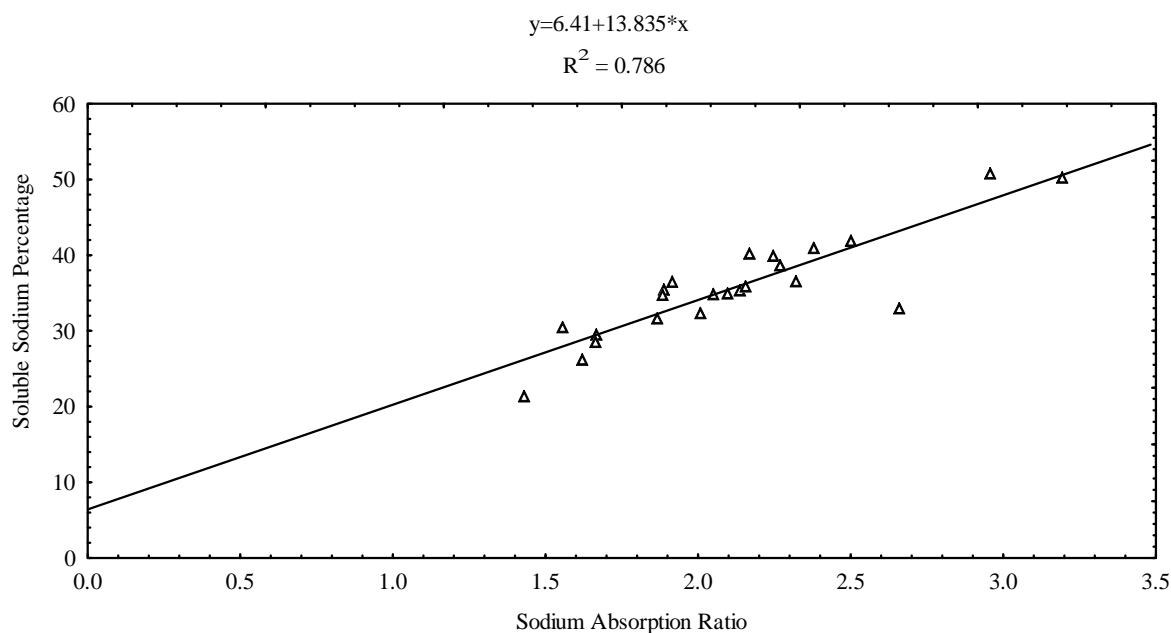
It is reported that salinity and sodicity are the principal water quality concerns in irrigated areas receiving such water [48]. Saline-sodic irrigation water, coupled with limited rainfall and high evaporation, may increase soil sodicity significantly. In general, when sodium is an important component of the salts, there can be a significant amount of adsorbed sodium making the soil sodic [31].



**Figure 2.** Rating of water samples in relation to salinity and sodium hazard.



**Figure 3. Correlation between sodium adsorption ratio and conductivity.**



**Figure 4. Correlation between sodium adsorption ratio and percentage sodium.**

The ratio of the exchangeable  $\text{Na}^+$  to total exchangeable cations (Exchangeable Sodium Percentage, ESP) is a good indicator for soil structure deterioration. Although, the ESP of 10-15% is generally accepted as a critical level, an ESP of 25% may have little effect on soil structure in a sandy soil, whereas an ESP of 5% is considered high particularly in soils containing 2:1 clay minerals

like montmorillonite [49]. The ESP of soils can be predicted quite well from the following the empirical relationship [30]:

$$\text{ESP} = \frac{100(-0.0126 + 0.01475\text{SAR})}{1 + (-0.0126 + 0.01475\text{SAR})} \quad (6)$$

The expected ESP for the experimental data would be



in range of 0.84-3.34 SAR, 2.16-4.97 adj SAR and 1.0-3.12 SAR RNA as shown in **Table 3**. The ESP predicted from adj SAR of treated wastewater was higher than those predicted from SAR and SAR RNA.

### 3.5. Chloride Hazard

The most common toxicity is from chloride ( $\text{Cl}^-$ ) in the irrigation water.  $\text{Cl}^-$  is not adsorbed or held back by soils, therefore it moves readily with the soil-water, is taken up by the crop, moves in the transpiration stream, and accumulates in the leaves. If the  $\text{Cl}^-$  concentration in the leaves exceeds the tolerance of the crop, injury symptoms develop such as leaf burn or drying of leaf tissue. Normally, plant injury occurs first at the leaf tips (which is common for chloride toxicity), and progresses from the tip back along the edges as severity increases. Excessive necrosis (dead tissue) is often accompanied by early leaf drop or defoliation [33]. The obtained  $\text{Cl}^-$  ion concentration of the samples varied from 171.44 to 254.92 mg/L (mean value = 205.25) representing slight to moderate degree of restriction on the use of this wastewater in irrigation [32]. While, according to USSL classification of irrigation water, the effluent samples can be used for moderately tolerant plants [30]. Chemical analysis of plant tissue is commonly used to confirm chloride toxicity. The part of the plant generally used for analysis varies with the crop, depending upon which of the available interpretative values is being followed. However, for irrigated areas, the chloride uptake depends not only on the water quality but also on the soil chloride, controlled by the amount of leaching that has taken place and the ability of the crop to exclude chloride. Crop tolerances to chloride are not nearly so well documented as crop tolerances to salinity [32]. On the other hand, significant correlation was found between  $\text{Na}^+$  and  $\text{Cl}^-$  of wastewater ( $R^2 = 0.60$ ), suggesting that the common source of these ions is salt dissolution. The possible sources of these ions were anthropogenic and natural.

### 3.6. Magnesium Hazard

Generally,  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  maintain a state of equilibrium in most waters. Both  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  ions are associated soil aggregation and friability, but they are also essential plant nutrients. High concentration of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  ions in irrigation water can increase soil pH, resulting in reducing of the availability of phosphorous [23]. Water containing  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  higher than 10 meq/L (200 mg/L) cannot be used in agriculture [50]. The observed results show that 60% of the samples have exceeded 200 mg/L. High correlation was found between  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  of wastewater ( $R^2 = 0.68$ ), suggesting that the common source of these ions is carbonate dissolution.

Another indicator that can be used to specify the magnesium hazard (MH) is proposed by reference [51] for irrigation water as in the following formula:

$$\text{MH} = \frac{\text{Mg}^{2+}}{\text{Ca}^{2+} + \text{Mg}^{2+}} \times 100 \quad (7)$$

where,  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  ions are expressed in meq/L.

If the value of MH is less than 50, then the water is safe and suitable for irrigation [50]. From the calculated value (**Table 3**), the MH values range between 7.97-56.53%, (mean = 39.86) and the treated wastewater can be classified with few exception as suitable for irrigation use.

### 3.7. Residual Sodium Carbonate (RSC)

The excess sum of  $\text{CO}_3^{2-}$  and  $\text{HCO}_3^-$  in wastewater over the sum of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  influences the unsuitability of wastewater for irrigation. In water having high concentration of  $\text{CO}_3^{2-}$  and  $\text{HCO}_3^-$ , there is tendency for  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  to precipitate as carbonates. To qualify this effect, an experimental parameter termed as RSC [29] was used. It can be calculated as follows:

$$\text{RSC} = (\text{CO}_3^{2-} + \text{HCO}_3^-) - (\text{Ca}^{2+} + \text{Mg}^{2+}) \quad (8)$$

All ion concentrations are reported in meq/L.

The water with high RSC has high pH and land irrigated by such waters becomes infertile owing to deposition of sodium carbonate as known from the black colour of the soil [29]. According to the USSL [30], RSC value less than 1.25 meq/L is safe for irrigation, a value between 1.25 and 2.5 meq/L is of permissible quality and a value more than 2.5 meq/L is unsuitable for irrigation. The calculated RSC value (-12.75) show that all samples have RSC less than zero and are good suitable for irrigation purposes.

### 3.8. Other Related Characteristics

The oxygen demand arises from the biochemical degradation of organic materials, the oxidation of inorganic material such as sulphides and ferrous and possibly the oxidation of reduced form of nitrogen [28]. The BOD, COD and TSS values in the present study varied from 12 to 66 mg/L, 36 to 80 mg/L and 10 to 112 mg/L, respectively. With few exceptions, the treated wastewater in this study area displayed higher values of BOD, COD and TSS. Calculated results highlight that the final effluent produced from Al-Rustamiyah WWTP did not meet the Iraqi National Standards set by Regulation 25 of 1967. Ultimately, reconsideration of the WWTPs system and completed environmental impact assessment are needed.

## 4. Conclusions

Interpretation of physical and chemical analysis revealed

that the treated wastewater of Baghdad City is slightly alkaline in nature. The US salinity diagram illustrates that most of the treated wastewater samples fall in the field of C3S1, indicating high salinity and low sodium water, which can be used for irrigation on almost all types of soil without danger of exchangeable sodium. Therefore, the sustainable use of treated wastewater in agriculture can be beneficial to the environment in such a way that minimizes the side effects on the quality of downstream water resources, but it requires the control of soil salinity at the field level.

Based on these results that proper management of wastewater irrigation and periodic monitoring of quality parameters are required to ensure successful, safe and long term reuse of wastewater for irrigation. It is recommended as a matter of high priority that treated wastewater is considered and made a reliable alternative source in water resources management. Agricultural wastewater reuse can effectively contribute to fill the increasing gap between water demand and water availability particularly in semi-arid areas. In future, further work is needed to examine organic and toxic constituents in wastewater and more intensive sampling and studies to measure any change of chemical elements in wastewater, irrigated soil and plant.

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# Exposure to Oil during Meiosis Results in Alterations in Meiotic Chromosomes that are Similar to Age-Related Changes in the Nematode *Caenorhabditis elegans*

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## ABSTRACT

Exposure of young *C. elegans* nematodes to three different concentrations of oil resulted in changes in the meiotic chromosomes, nucleus, nucleolus, and nuclear envelope associations. Such alterations decreased the viability and fertility of this organism which was used as a biological model. The morphological changes in the “young” group were similar to nematodes that were senescent and post-reproductive. Comparison of meiotic chromosomes at the pachytene stage of meiosis from young, old, and oil-exposed wild-type hermaphrodites were made following three-dimensional electron microscopy reconstruction of serial ultrathin sections. Age-related and oil-exposure related changes included: 1) Induced condensation of chromatin with increased variance in length of chromosomes; 2) Changes in nuclear and nucleolar volume; 3) Increased density of the nucleoplasm; and 4) Absence of Disjunction Regulator Regions, resulting in the loss of control of the segregation of the X-chromosome into gametes during meiosis. Abnormal clustering of the telomeric ends of the chromosomes was present on the nuclear envelope affecting the segregation of the chromosomes during meiosis.

**Keywords:** Oil, Age, Chromosome, Synaptonemal Complex

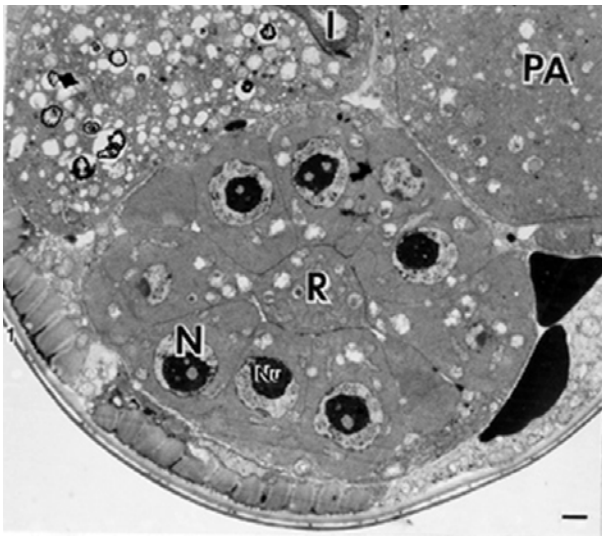
## 1. Introduction

Cytogenetic studies in many organisms have shown that there is a change in chromosome number or an increase in structural chromosome alterations associated with aging [1]. Such changes would account for the cellular deterioration characteristic of senescent tissues since the production of gene products would be altered. It is of significance that such changes in normal structure and function can be induced by exposure to oil [2].

In the present study, the free-living nematode *Caenorhabditis elegans*, was exposed to varying concentrations of oil and used as a biological model to characterize changes associates with such exposure. This nematode is ubiquitous and is found in virtually all types of environments, including marine. It reproduces primarily as a self-fertilizing hermaphrodite, but a small number of morphologically distinct males (0.3%) are also present in the population [3]. The adult hermaphrodite has a pair of

ovaries, 810 nongonadal nuclei and five pairs of autosomes with two X chromosomes (2A:XX). The pachytene nuclei are arranged peripherally around a central rachis (**Figure 1**). The adult male has a single testis, 970 nongonadal nuclei, and five pairs of autosomes but only a single X chromosome (2A:XO) [4]. Males arise from gametes that have been produced after X-chromosome nondisjunction, thus, the two sexes experience an unequal number of X chromosomes. They must compensate for this state of aneuploidy and develop mechanisms for gene expression and dosage compensation [5]. Distinct structures on the chromosomes called “Disjunction Regulator Regions” control the separation of the X chromosome during meiosis. Such separation is essential in order to produce functional gametes that have the correct number of chromosomes [6].

The synaptonemal complex (SC) is a tripartite, proteinaceous structure that is found between paired chromosomes during the pachytene stage of meiosis. It has



**Figure 1.** Cross-section of the gonad from *C. elegans*. The meiotic nuclei (N) of the oocytes at the pachytene stage of meiosis are arranged peripherally around a central rachis (R). Nucleolus (Nu) Proximal arm (PA) of the gonad. Bar equals 0.5  $\mu$ m.

been highly conserved throughout evolution and occurs in a wide variety of organisms [7]. Its presumed role is twofold: 1) Maintenance of proximity of homologous chromosomal segments, whereby the axial cores of the homologs become the lateral elements of the SC [8]; and 2) Regulation of ordered meiotic disjunction in which case the SC is maintained at the chiasma [9]. Irregular nondisjunction of the chromosomes at anaphase I may be a reflection of the pairing pattern at pachytene such that multivalent associations, as in polysomies and duplications, yield complex configurations that inhibit segregation [10]. In systems in which normal bivalent formation has been altered by the presence of chromosomal aberrations, the resultant univalents do not show ordered segregation patterns, and the resultant gametes are aneuploid.

In *C. elegans*, there are six SCs per germline nucleus. The XX hermaphrodite has five SCs associated with the autosomal bivalents and one SC for the XX bivalent. The XO male has only five SCs since the univalent X chromosome remains heterochromatic during pachytene [11,12]. The tripartite SC consists of two lateral elements and a striated central element with a distance between axes of approximately 100 nm. One end of the SC is attached to the inner nuclear envelope while the other end remains free in the nucleoplasm [12]. There is no evidence of the configuration known as the “bouquet” which facilitates homologous pairing via restricted chromosome movement [13], thus the ends of the chro-

mosomes are randomly attached around the nuclear envelope. In addition, there are no recombination nodules present in *C. elegans* although they have been described in other organisms [14].

Disjunction Regulator Regions (DRR) were first described in *C. elegans* [11,12]. Statistical analysis of the occurrence of DRRs and the frequency of X-chromosome nondisjunction support the observation that there is a correlation between the presence of DRRs and normal disjunction of the X chromosome [6], which is required for the production of functional gametes.

The American Petroleum Institute’s Manifesto on Crude Oil (CAS # 8002-05-09; (a.k.a. Sweet Crude, Arab Medium, West Texas Inter-Cushing, Earth Oil, Petroleum Oil, Rock Oil, Zafiro) defines the composition of Crude Oil as “A complex combination of hydrocarbons”. It consists predominantly of aliphatic, alicyclic and aromatic hydrocarbons. It may also contain small amounts of nitrogen, oxygen and sulfur compounds. Specifically Crude Oil (CAS # 8002-05-9) contains Benzene, Butane, N-Hexane, Isopentane, Pentane, and Stoddard Solvent. Benzene is a known human carcinogen and is identified by NTP, OSHA, and IARC as a Group 1 carcinogen. This category encompasses light, medium, and heavy petroleum, as well as the oils extracted from tar sands. Along with the numerous oil spills that are known, there are also significant leaks and seepages into the environment that are not reported that are equally hazardous to the environment and negatively affect the ecosystem. As early as 1982, cytogenetic effects, including mitotic sister chromatid breaks and chromosome damage, were reported after exposure to oil [2].

This study represents the first examination of the changes in nuclear architecture and the meiotic chromosomes after exposure to oil. Serial ultrathin sections and electron microscopy were used to provide three-dimensional analysis of the relationships of all nuclear components and identify changes correlated to the cytotoxicity of oil.

## 2. Materials and Methods

The *C. elegans* individuals studied were hermaphrodites received from the Caenorhabditis Genetics Center. The worms were grown in agar plates made with 5%, 25%, and 50% Texas Crude oil in the form of an emulsion which they moved through at all levels of the agar. The plate was seeded with OP50 *E. coli* bacteria as a food source. In order to obtain worms at specific ages, adult worms were removed from the plates leaving only first stage larvae. Worms were selected at 4-5 days and 8-9 days of age, thus, forming the “young” and “old” groups and were processed for electron microscopy as previously

described [11,15].

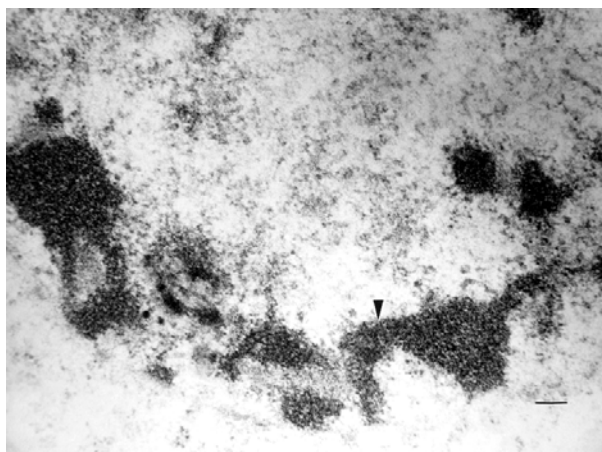
The pachytene karyotypes from analysis of synaptonemal complexes of five early-mid pachytene stage nuclei from worms that were 4-5 days old (“young”), 8-9 days old (“old”), and 5%, 25% and 50% oil-exposed were determined using the Metamorphosis program [16]. Nuclear and nucleolar volumes were also determined using the Metamorphosis program. A total of 25 pachytene karyotypes were analyzed.

### 3. Results and Discussion

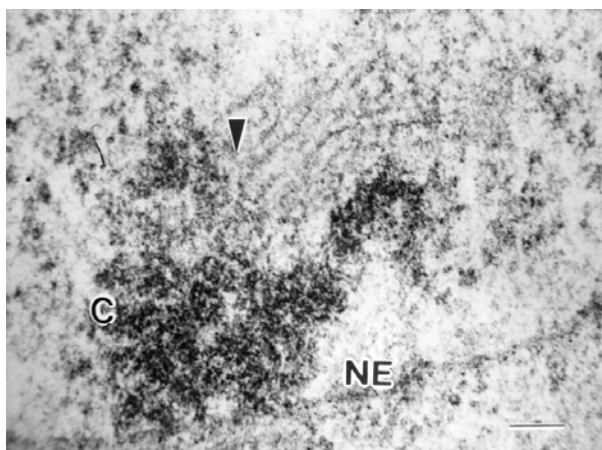
Comparison of the synaptonemal complexes from the meiotic chromosomes at the stage of pachytene, revealed significant changes in the chromatin, the nucleus, and its associated components (**Table 1**). These included: 1) Condensation of chromatin (more condensed in older specimens and those exposed to oil; **Figure 2**); 2) Decrease in length of the chromosomes due to condensation; 3) Decrease in volume of the nucleus due to changes in the nuclear matrix; and 4) Alterations in the association of the telomeric ends of the chromosomes with the nuclear envelope, such that the normally random attachment of the telomeres was restricted to a small region of the nucleus. This resulted in a clustering of the ends of the chromosomes, which affects their ability to segregate properly into the gametes. In this organism, the clustering of the chromosome ends may promote entanglements which cannot be easily resolved. In addition, the nucleus of the old and oil-exposed individuals, accumulated electron-dense proteinaceous materials, and the nuclear envelope was irregular. These are manifestations of the degradation of the nucleoplasm and protein matrix.

The synaptonemal complexes (SC), which are normally tripartite proteinaceous structures within the meiotic chromosomes, were altered in the ten selected worms exposed to 25% and 50%, such that the structure of the SCs became indistinct and the lateral elements and the central elements were not discernible (**Figure 3**). This is compared to normal SCs where the two lateral elements were clearly visible (**Figure 4**). In addition, there was a 26% decrease in synaptonemal complex length comparing young worms to those five selected worms exposed to 50% oil (**Figure 5, Table 1**). There was a variation in length of plus or minus of two percent from one worm to the next. This may be the result of the abnormal condensation of the chromatin which resulted in altered SC structure. The SC is required for pairing and separation of the chromosomes during meiosis, thus, alteration in its structure may result in decreased viability and fecundity.

The Nuclear Volume decreased by 37% from the five young worms and 18% from the five old worms to the five worms selected which were exposed to 50% oil.



**Figure 2.** *C. elegans* 4-5 days old (“young”), exposed to 50% oil, demonstrate the same condensation in chromatin (arrow) as in senescent worms. Bar equals 0.1  $\mu$ m.



**Figure 3.** Synaptonemal complexes (arrow) from 4-5 day old (“young”) *C. elegans* exposed to 50% oil are altered in structure. The lateral elements and central element are not discernible. Nuclear Envelope (NE) Chromatin (C) Bar equals 0.2  $\mu$ m.

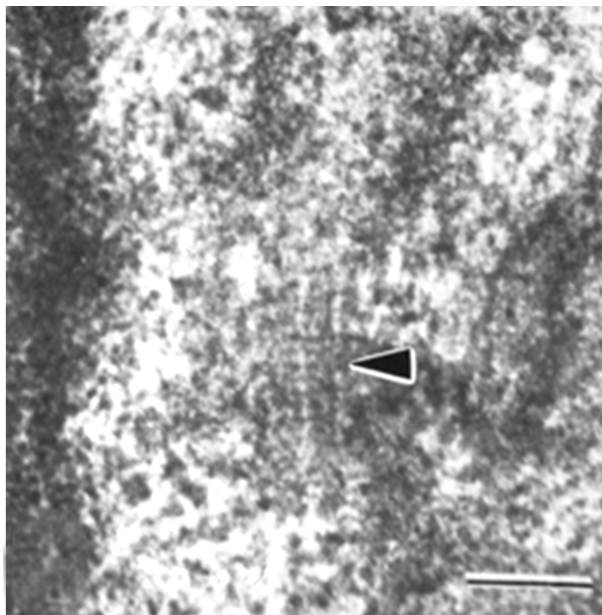
Nuclear volume also decreased 13% between those worms exposed to 5% versus 50% oil (**Figure 6, Table 1**). This was due to abnormal condensation, *i.e.* heterochromatinization, of the chromosomes and altered protein structure. Heterochromatinization is a normal process in senescent cells (those from *C. elegans* that are 8-9 days old) and results in decreased expression of genes. The fact that the same morphology is manifested in 4-5 day old cells (“young”) after exposure to 50% oil, is significant, as it indicates the deterioration and loss of function in the oocytes [17].

Disjunction Regulator Regions (DRR) appeared as decondensed chromatin and were associated with the synaptonemal complex. In *C. elegans*, they have been shown

**Table 1. Average pachytene chromosome lengths of *C. elegans* wild-type from young, old, and oil-exposed individuals from three different concentrations by reconstruction of synaptonemal complexes. All chromosome lengths are in microns.**

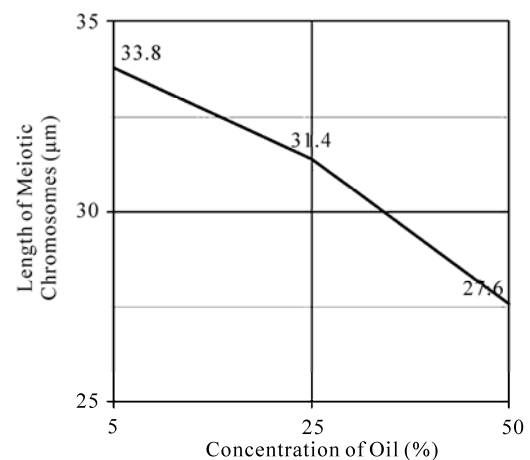
SC #	Young (4-5 days old)	Old 8-9 days old)	Oil-Exposed (5% Oil)	Oil-Exposed (25% Oil)	Oil-Exposed (50% Oil)
1x	2.5	2.4	2.4	2.2	1.9
2	4.5	4.1	4.1	4.0	3.8
3	6.0	5.5	5.3	5.0	4.7
4	6.2	6.0	6.0	5.4	4.7
5	7.9	7.1	7.0	6.8	6.5
6	10.4 <sup>a</sup>	9.1 <sup>a</sup>	9.0 <sup>a</sup>	8.0 <sup>a</sup>	6.0 <sup>a</sup>
Total	37.5	34.2	33.8	31.4	27.6
Nuclear Volume $\mu\text{m}^3$	12.4	9.4	9.0	8.8	7.8
Nucleolar Volume $\mu\text{m}^3$	5.6	4.5	4.5	4.4	4.2
% Nu Vol.	45.2	47.8	50.0	50.0	58.3
# DRR	6	3	0	0	0
Position of NOR from Telomere	18%	25%	22%	19%	37%

(a) Nucleolar Organizer Region (NOR) found on this chromosome; (x) X-chromosome



**Figure 4. Normal synaptonemal complex from a 4-5 day old (“young”) *C. elegans* comprised of two lateral elements (arrow) and a striated central element that is formed between them. Bar equals 0.1  $\mu\text{m}$ .**

to be responsible for controlled segregation of the X-chromosome during meiosis [6]. The average number of DRRs in nuclei from 4-5 day old nematodes was 6,

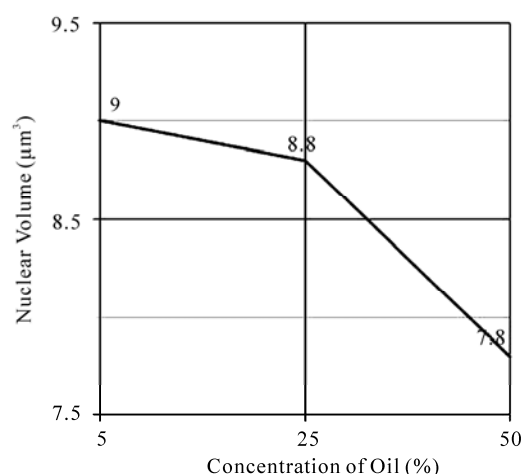


**Figure 5. Change in meiotic chromosome length after exposure to varying concentrations of oil.**

whereas after they have been exposed to 50% oil, there were none. Thus, segregation of the X-chromosome was no longer under the proper control and unequal numbers of chromosomes would be distributed to the gametes. Such aneuploid gametes have decreased function.

This study was done in order to determine the effects of exposure to oil on meiotic chromosomes and nuclear components. The nematode *C. elegans* was used as a biological model, however, the mechanisms of meiosis are conserved throughout evolution and these aberrations





**Figure 6.** Change in meiotic nuclear volume after exposure to varying concentrations of oil.

may pertain to humans as well.

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# Hazard Assessment of Alternatives to Dicofol

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## ABSTRACT

*Dicofol was listed by International POPs Elimination Network (IPEN) as requiring immediate and urgent consideration and thus was considered as a new candidate by Persistent Organic Pollutant Review Committee (POPRC) as a possible persistent organic pollutant (POP). Dicofol is structurally similar to DDT. It is persistent in food and water, highly toxic to aquatic life and causes egg-shell thinning in some bird species. High concern, due to the lack of dicofol measurements in the Arctic, proving long range transport and bioaccumulation in wild life species, supports further impact assessment of this product. Under Stockholm Convention, substances identified as POPs are regulated with the objective to protect the environment and the human health. According to this objective, the search of environmental and healthy alternatives is helpful. This paper discusses the use of three groups of chemicals currently applied as alternatives to dicofol. An exhaustive review of the synthesis of dicofol, starting from DDT, and compared to possible substitutes is presented: 1) active principle with fluoralkenyl are proposed as an environmental and healthy alternative to dicofol, 2) inhibitor agents of mitochondrial electron transport as chlorfenapyr, hydramethylnon and pyridaben and 3) pesticides commonly applied in agricultural practices as oxythioquinox, fenbutatin-oxide and formetanate hydrochloride.*

**Keywords:** Dicofol, POPs, Chemical Structure, Effects, Alternatives

## 1. Introduction

Dicofol (1,1'-bis(*p*-chlorophenyl)-2,2,2-trichloro-ethanol) is an organochlorine compound, miticide and acaricide. It is applied in a wide variety of crops, fruits, vegetables, ornamental and field crops. The use of this product is extended on more than 30 countries, and on more than 60 different crops (aprox. 0.4 to 3.0 Kg a.i./ha) [1].

The worldwide consumption of dicofol is 2750 tons/year as the following: 290 tons/year in Western Europe, 180 tons/year in Africa and Western Asia, 1820 tons/year in Asia, 170 tons/year in South America and 290 tons/year in North America. In Spain the use is 100-150 tons/year [2].

Dicofol production was temporarily banned by U.S. EPA in 1986. Afterwards it was reinstated as consequence of a new manufacturing process which produced technical-grade dicofol (< 0.1% DDT, DDT and related substances). DDT level in dicofol can't exceed 0.1% in Canada.

The use of dicofol is allowed in several countries of the EU (451/2000) [3]. Council Directive 79/117/EEC prohibits the use and marketing of products containing less than 78% *p,p'*-dicofol or more than 1 g/kg (= 0.1%) of DDT.

Persistent, bioaccumulative and toxic substances (PBTs) and Persistent Organic Pollutants (POPs) are identified or addressed through various national, regional and global initiatives [4]. In the Stockholm Convention framework, the assessment of POPs is described as an evaluation of whether the chemical is likely, to lead to significant adverse human health and/or environmental effects, as a result of its long range environmental transport, so that global action is warranted.

Dicofol is currently under a review process for its designation as POP under the Stockholm Convention. Dicofol meets POP criteria but further assessment is needed regarding ecotoxicity of metabolites, monitoring in remote areas of dicofol and its metabolites [5].

Dicofol is degraded in water, sediments and soil. In water, *p,p'*-dicofol meets the persistence criteria with a half life of two months at pH = 5; *o,p'*-isomer has a half life of 47, 0.3 and < 0.1 days at pH 5, 7 and 9 respectively; *p,p'*-isomer has a half life of 85, 4 and < 0.1 days at pH 5, 7 and 9 respectively. In sediments, isomers *o,p'*- and *p,p'*- (pH of aqueous phase between 7.6 and 7.8) fail the persistence criteria, as the half life is lower than one day. The half life of dicofol metabolites in sediments is between 7 and 429 days. In soils, half life of the *o,p'*- and *p,p'*-isomer is between 8 and 35 days, and between 21 and 60 days, respectively [5].

The mechanism of bioaccumulation of dicofol is similar to other POPs. Dicofol has a log Kow of 4.08-5.02 and a bioconcentration factor in fish of 8050-13000.

Due to its vapour pressure (1 Pa) and estimated half life in atmosphere (3.1 days), dicofol is expected to volatilise significantly and it is assumed to be transported in the atmosphere. Up to now, it is worthy of note that no monitoring data are available in remote areas. It has been hypothesized that dicofol may be globally distributed and should meet criterion for potential long-range atmospheric transport.

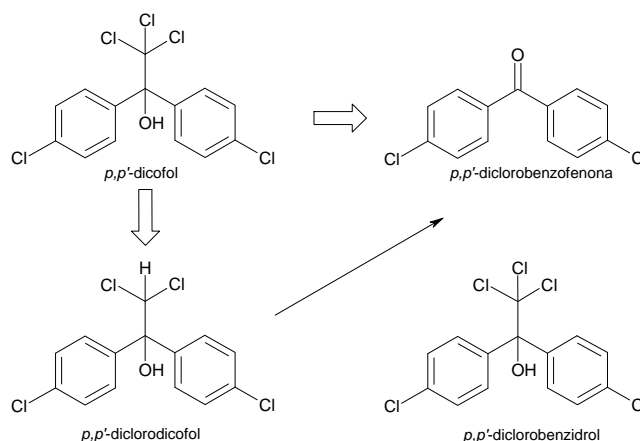
Dicofol is fairly toxic to mammals but isn't carcinogenic. It is reported to be repro-toxic in wildlife, and it may reduce eggshell quality as well. Dicofol is very toxic for aquatic organisms with lethal/effective concentration,  $L(E)C_{50}$  values of 15-120  $\mu\text{g/l}$  obtained by acute toxicity tests and, no observed effect concentration, NOEC values of 4.4-125  $\mu\text{g/l}$  for chronic effects. Evidence of induction hepatic microsomal metabolism is available for rats [6]. Metabolites show similar toxic effects for aquatic organism based on range-finding studies and quantity structure-activity relationship (QSAR) model estimations. In **Scheme 1**, degradation (biotic and abiotic) pathways of dicofol and metabolites are shown [7].

This work shows a review of possible substitution alternatives to dicofol from the chemical perspective, analyzing the product synthesis, its chemical structure and its effects on the environment and human health. Target chemical groups in this article are: 1) active principles such as fluoralkenyl derivatives introduced as an environmental and healthy solution to dicofol, 2) second group based on the mode of action, mitochondrial electron transport inhibitors as chlorfenapyr, hydramethylnon and pyridaben; and 3) alternative pesticides commonly used in agricultural practices as oxythioquinox, fenbutatin-oxide and formetanate hydrochloride.

## 2. Chemistry Facts

### 2.1. DDT and Dicofol Synthetic Processes

DDT is produced as an intermediate in dicofol synthesis.



**Scheme 1. Metabolite pathway in dicofol transformation.** Reaction pathways shown are from Brown & Casida (1987). Arrow thickness is added to roughly indicate relative rates of conversion.

The active ingredient dicofol is a mixture of approximately 80% of *p,p'*-dicofol and 20% of *o,p'*-dicofol [8]. It is produced by hydroxylation reaction of DDT, which is an emulsionable concentrate and can be commercialized as wettable tablets or water-soluble.

DDT was first synthesized in 1874 by Zeidler and its insecticidal properties were discovered by J.R. Geigy. The formation of technical DDT was optimized by Mosher, improving the original synthesis by Zeidler and the variation of Baeyer [9], **Scheme 2**.

The reaction rate at low temperatures is slow, and at high temperatures results on the degradation of the product, although this can be avoided by using major acid concentration and lower temperature, or by using diluted acid and higher temperatures. A secondary reaction it is always raised, that supposes the chlorobenzene sulfonation. At last, the higher DDT yield is obtained at 90°C, with a 98% sulphuric acid concentration, and a 1:4 chlorobenzene excess, stirring for 8 h at 5-10°C, DDT yield is 97%.

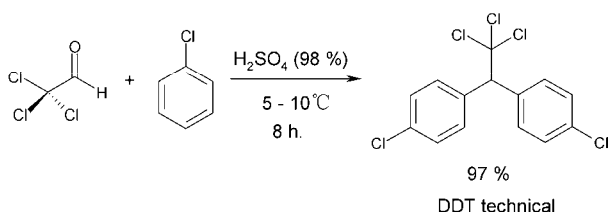
Technical DDT contains approximately 77% of *p,p'*-DDT, 15% of *o,p'*-DDT and 1.5% of an oily compound that is 2-trichloro-1-*p*-chloro-phenylethanol.

DDT synthesis was optimized using fluorhydric acid as condensing agent [10], **Scheme 3**.

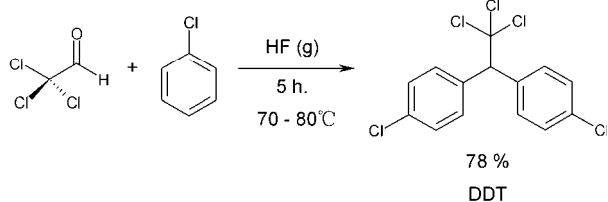
Latterly, DDT analogues synthesis has been prepared to examine its insecticidal properties [11].

Dicofol is produced from chloral (trichloroacetic aldehyde), monochlorobenzene and oleum (fuming sulphuric acid: sulphur VI containing excess of sulphur trioxide), **Scheme 4**.

Another synthetic process used to obtain technical dicofol was developed by Tang [12], it starts from DDT



Scheme 2. Preparation of technical DDT.



Scheme 3. Improved preparation of technical DDT.

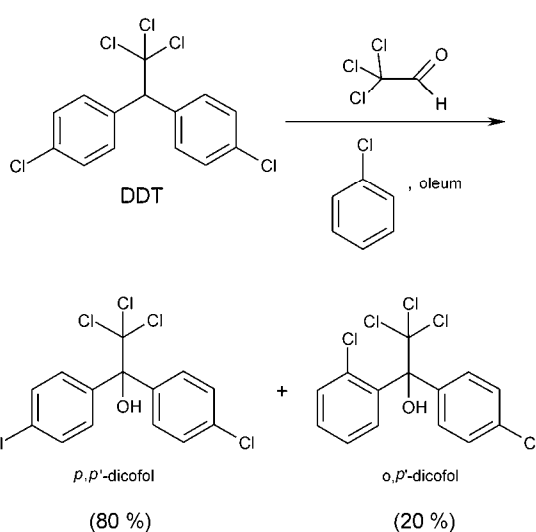
and after chlorination and hydrolysis the desired product is obtained, **Scheme 5**. Impurities generated throughout the synthetic process are DDT and Cl-DDT.

## 2.2. Alternative Products

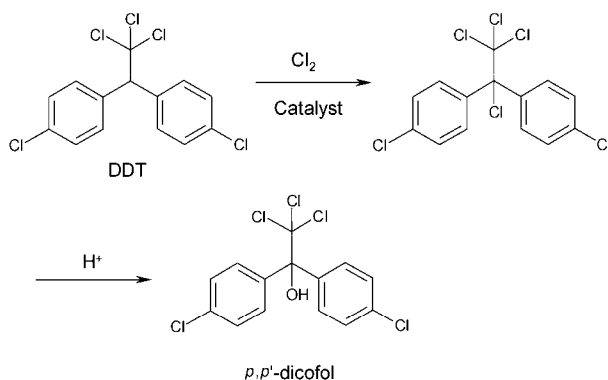
The pesticidal natural action of a compound is predominantly associated with its structure [13]. Also, the different moieties attached to parent compound, their spatial arrangements within the molecule, nature of substituents, polarity, symmetry and asymmetry of molecules, the solubility, sorption values, etc., have a direct or indirect bearing on the toxicity of the parent pesticidal compound. So, having an insight into the structure and toxicity relationship within each class of pesticides provides a better understanding of this correlation. The understanding of this relationship is vital in order to generate a molecule with a tailored fragment powered to act on the pests.

### 2.2.1. Fluoroalkenyl Derivatives

Fluoroalkenyls [14], including all the geometric and stereoisomers, N-oxides, and salts thereof, can be an option to dicofol. These are compounds of **Figure 1** wherein X is H, F, C1-C4 haloalkyl, A is O, S or NR<sup>1</sup>; B is C1-C4 alkylene; Y is a 5- or 6-membered heteroaromatic ring or an aromatic 8-, 9- or 19-membered fused heterobicyclic ring system, each ring or ring system optionally substituted with 1 to 6 substituents independently selected from R<sup>2</sup>, or Y is O(CH<sub>2</sub>CH<sub>2</sub>O)mR<sup>3</sup>; and R<sup>1</sup>, R<sup>2</sup> and R<sup>3</sup>, n and m are as defined in the figure. These products have showed extremely effective for controlling invertebrate pest comprising contacting the invertebrate pest or its environment, thus an amount of a fluoroalkenyl compound is conjugated with an amount of at least one additional biologically active compound or agent.



Scheme 4. Synthesis of dicofol starting from DDT.



Scheme 5. Alternative synthesis of dicofol starting from DDT.

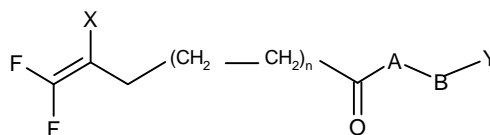


Figure 1. Fluoroalkenyl derivatives.

These additional biologically active compounds are showed on **Table 1**.

The mode of action of the pesticide in target organism is closely associated with the structure of the pesticidal compound. The structure of the parent molecule of structure is not only responsible for the activity but also the nature of substituents, or also the presence of an epoxide ring, double-triple bond, conjugation, aromaticity and stereochemistry determine the toxicity of the pesticidal compound. So, understanding of the structure of

**Table 1. Control agents for invertebrate pest based on its mode of action.**

Mode of Action	Control Active Principle
Sodium channel modulators	Bifenthrin, cypermethrin, cyhalothrin, lambda-cyhalothrin, cyfluthrin, beta-cyfluthrin, deltamethrin, dimefluthrin, esfenvalerate, fenvalerate, indoxacarb, metofluthrin, profluthrin, pyrethrin and tralomethrin.
Acetyl cholinesterase inhibitors	Chlorpyrifos, methomyl, oxamyl, thiodicarb, formetanate hydrochloride and triazamate.
Neocotinic receptor modulator	Acetamiprid, clothianidin, dinotefuran, imidacloprid, nitenpyram, nithiazine, thiacloprid and thiamethosam.
Insecticidal macrocycles lactones (Gamma-aminobutyric acid)-regulated chloride channel modulators [GABA]	Spinetoram, spinosad, abamectin, avermectin and emamectin.
Chitin synthesis inhibitor	Endosulfan, dicofof, ethiprole and fipronil.
Juvenile hormone mimics	Buprofezin, cyrimazine, flufenosuron, hexaflumuron, lufenuron, novaluron, noviflumuron and triflumuron.
Inhibitors of oxidative phosphorylation, disrupters of ATP formation (inhibitors of ATP synthase)	Diofenolan, fenoxycarb, methoprene and pyriproxyfen.
Octapamine receptor modulators	Fenbutatin-oxide.
Ecdysone receptor agonists	Amitraz.
Ryanodine receptor ligands	Azadirachtin, methosyfenozide and tebufenozide.
Nereistoxin analogs	Ryanodine, anthranilic diamides such as chloroantraniliprole and flubendiamide.
Mitochondrial electron transport inhibitors	Cartap.
Lipid biosynthesis inhibitors	Chlorfenapyr, hydramethylnon and pyridaben.
Disrupting protein function	Spirodiclofen and spiromesifen.
Cyclodiene insecticides	Oxythioquinox.
Nucleopolyhedrovirus mixture	Dieldrin, cyflumetofen, fenothiocarb, flonicamid, metaflumizone, pyrafluprole, pyridalyl, pyripole, spirotetramat and thiosultap-sodium.
	HzNPV and AfNPV. Bacillus thuringiensis and encapsulated delta-endotoxins or Bacillus thuringiensis such as Celicap, MPV and MPVII, as well as naturally occurring and genetically modified viral insecticides including members of the family Baculoviridae as well as entomophagous fungi.

compounds and their correlation with toxicity to target organism is a very important parameter for developing better designed pesticidal compounds with tailored toxicidal properties on different pests.

These invertebrate pests can be arthropods (e.g. insects, mites, spiders, scorpions, centipedes, millipedes, pill bugs and symphylans, etc.), gastropods (e.g. snail, slugs, etc.) and nematodes (e.g. worms, etc.).

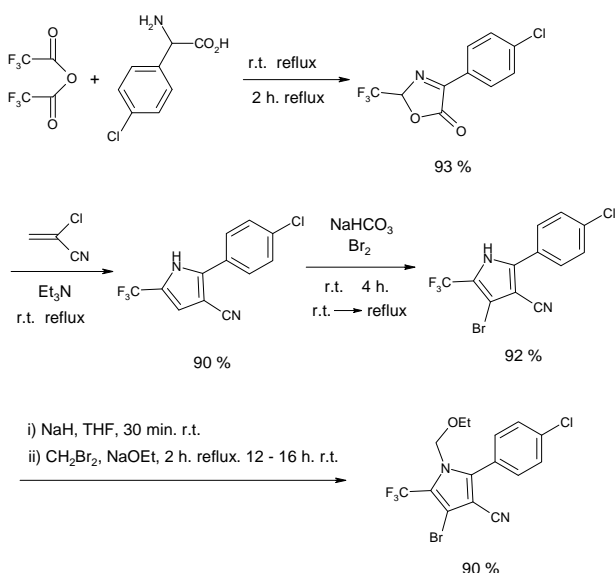
These compounds can generally be used as a formulation or a composition with a carrier suitable for agronomic or nonagronomic uses comprising at least one of a liquid diluent, a solid diluent or a surfactant. The formulation or composition ingredients are selected to be consistent with the physical properties of the active ingredient, mode of application and environmental factors such as soil type, moisture and temperature. Useful formulations include liquids such as solutions (including emulsifiable concentrates), suspensions, emulsions (including microemulsions and/or suspoemulsions) and the like which optionally can be thickened into gel; others include solids such as dusts, powders, granules, pellets, tablets, films (including seed coatings), and the like which can be water-dispersible ("wetttable") or water-soluble. Encapsulation can control or delay release of the active ingredient. Sprayable formulations can be extended in suitable media and used at spray volumes from about one to several hundred liters per hectare.

### 2.2.2. Inhibition Agents Acting as Mitochondrial Electron Transport

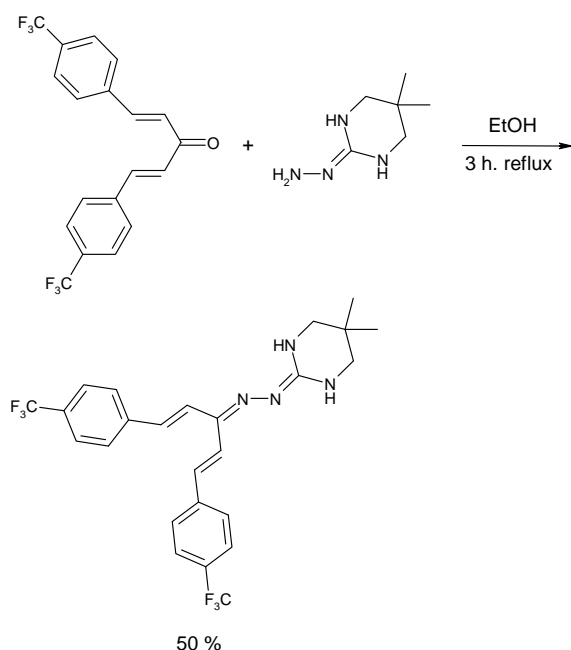
Pardini *et al.* [15] have determined that dicofof acts as a mitochondrial electron transport inhibitor, studies suggest that some organochlorine pesticides and the hydroxylated breakdown products of carbaryl are inhibitory towards mitochondrial electron transport systems *in vitro*. Further investigations should be conducted to determine if the inhibition of mitochondrial electron transport systems is of toxicological importance in the intact organism. Consequently, dicofof ought to be compared with products that have the same mode of action, such as chlorfenapyr, hydramethylnon and pyridaben.

Chlorfenapyr synthesis was developed by Xu [16] starting from *α-p*-chlorophenyl glycine, **Scheme 6**. Glycine reacts with trifluoroacetic anhydride, and latterly with 2-chloroacrilonitrile, giving an intermediate that is 2-(*p*-chlorophenyl)-5-(trifluoromethyl)pyrrol-3-carbonitrile. This intermediate reacts through a bromination reaction at pyrrol ring in the presence of a weak base, and the resulting bromide performs an ethoxymethylation with methyl dibromide and sodium ethoxide, total yield is 65%. Chlorfenapyr purity is 95%, and the bromination process has been improved significantly.

Hydramethylnon synthesis was achieved through the coupling in alcoholic media refluxing the compound 1,5-bis[*p*-(trifluoromethoxy)phenyl]-1,4-pentadien-3-one afforded the desired product in 50% yield [17], **Scheme 7**.



Scheme 6. Synthesis of chlorfenapyr.



Scheme 7. Synthesis of hydramethylnon.

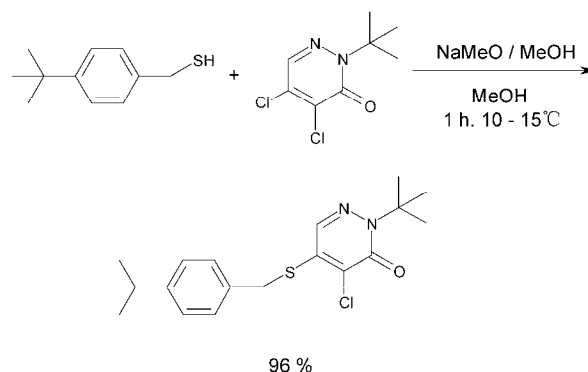
Pyridaben synthesis was developed by Xu [18] starting from 2-*tert*-butyl-4,4-dichloropyridazin-3(2H)-one, and 4-*tert*-(butylphenyl)methanethiol, in sodium methoxide media, stirring for one hour at controlled temperature, obtaining the final product in 96% yield, **Scheme 8**.

**2.2.3 Pesticide Alternatives to Dicofol of Common Use**  
Other pesticides analyzed due to its major use in different crops are oxythioquinox, fenbutatin-oxide and formetanate hydrochloride [19]. These alternatives are more poi-

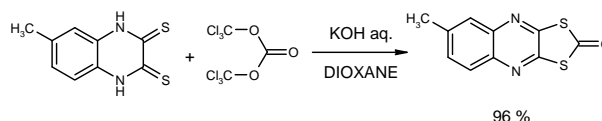
sonous than dicofol to beneficial insects, but many trials suggest that the efficacy is superior even though the production lost is about three percent. Oxythioquinox is a sulphur containing chemical, this quinoxaline-2,3-dithiocarbonate is obtained reacting a quinoxaline-2,3-dithiol substituted with an alkyl group with a carbonylating agent selected from triphosgene, and diphosgene. It is preferable that the quantity of the carbonylating agent is 0.34-5 molar times, for triphosgen, or 0.5-5 molar times, for diphosgene. The above reaction is better conducted under basic conditions to scavenge hydrochloric acid produced as by-product. The reaction temperature has to be maintained from  $-78$  to  $50^{\circ}\text{C}$  [20], **Scheme 9**.

An alternative synthesis to obtain this product starts from 2,6-dimercapto-6-methylquinoxaline that reacts with an hydroxide or the alike of an alkali metal in water or an alcohol solvent, and an organic solvent such as aromatic hydrocarbons, for example, toluene or xylene is added to the reaction mixture. The reaction mixture with the organic solvent is subjected to an azeotropic dehydration and a solvent replacement to form an alkali salt, and the alkali salt is reacted with the intermediated, where R is a methyl group preferably in an amount of 1.0-4.0 molar times in the presence of a phase transfer catalyst (e.g. a quaternary ammonium salt or a pyridinium salt) in an amount of 1-5% mol% at  $0-150^{\circ}\text{C}$  for 0.5-10 h. to provide the objective compound S,S-(6-methylquinoxaline-2,3-diyl)dithiocarbonate [21], **Scheme 10**.

Fenbutatin-oxide is an organotin compound, the synthesis of this compound is achieved starting from neophyl chloride, it occurs through a magnesium intermediate that reacts with tin tetrachloride, hydrolysis adding



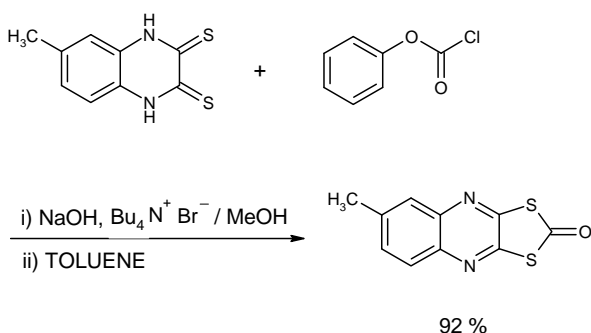
Scheme 8. Synthesis of pyridaben.



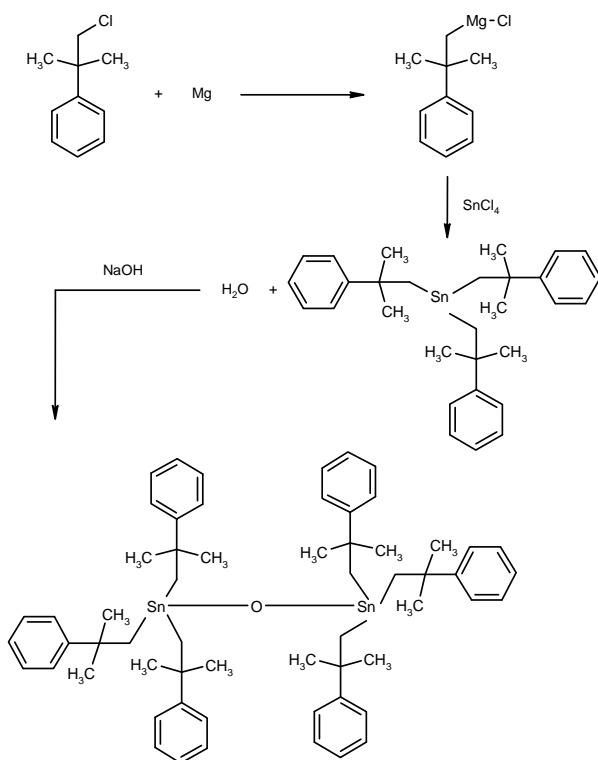
Scheme 9. Synthesis of oxythioquinox.

sodium hydroxide provides the final product [22], **Scheme 11**.

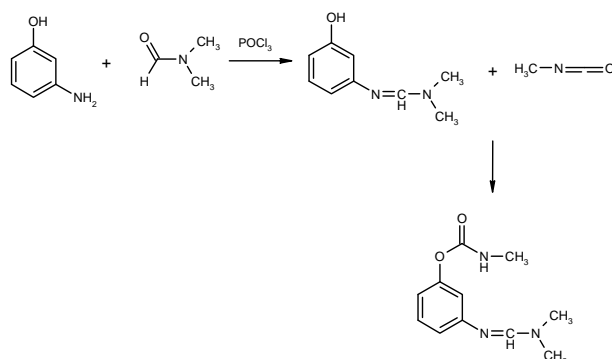
Formetanate hydrochloride is a bifunctional pesticide, so the two reactive groups of this molecule are formamidine and carbamate, and this remarks the water solubility, and also the toxicity and potential mobility in aqueous environments. The formetanate is obtained from *meta*-aminophenol and dimethylformamide in phosphorous oxychloride, after purification of the intermediate and reaction with methyl isocyanate and acid treatment produce the formetanate [23], **Scheme 12**. The final step in the synthesis may be accomplished by treating the formetanate with the appropriate acid chloride or anhydride in an inert solvent as diethyl ether [24].



**Scheme 10. Alternative synthesis of oxythioquinox.**



**Scheme 11. Synthesis of fenbutatin-oxide.**



**Scheme 12. Synthesis of formetanate.**

These anthropogenic compounds have some structural similarities, due to the relation structure-toxicity; most of them are organochlorines pesticides.

Dicolol is a chlorinated compound as well as chlorfenapyr and pyridaben, similarly dicolol shows a trichloromethyl group, and chlorfenapyr and hydramethylnon have a trifluoromethyl group, in most cases fluor enlarge the activity of a biologically active compound. Sulphur is contained in pyridaben and oxythioquinox, as historically use of pure sulphur to control insect pests.

Diazacompounds exhibit also activity as pyrimidine in hydramethylnon, pyridazine in pyridaben and a piperazine moiety in oxythioquinox. Another hit that increase the toxicity can be explained by the *para*-substitution, as in dicolol, hydramethylnon, pyridaben and oxythioquinox. Toxicity in fenbutatin-oxide can be explained by the metal element, since organo tin substances are known to disturb growth, reproduction, enzymatic systems and feeding patterns of aquatic organism.

Triethyltin is the most dangerous organo tin substance for humans. It has relatively short hydrogen bonds. When hydrogen bonds grow longer a tin substance will be less dangerous to human health. So that is why fenbutatin-oxide increases volume with a phenyl group. The dimmer explains double amount of toxic substance. The molecule geometry is important, as DDT and dicolol have a Z group of sufficient steric size, e.g. trichloromethyl, to inhibit the free rotation of the planar phenyl rings so that they are constrained to positions of minimum steric grouping, termed a trihedral configuration. Other symmetrical molecules are hydramethylnon and oxythioquinox (except methyl substitution that can be here considered as a minor group, since this group does not interfere with the C<sub>2</sub> operation).

Formetanate hydrochloride has two possible active moieties, is both arylformamidine and arylcarbamate groups, it appears to exert its toxicity to rats, houseflies, and mites as an anticholinesterase agent than a formamide [25], carbamates inhibit cholinesterase and prevent the termination



of nerve impulse transmission. The formetanate salt must be prepared to improved aqueous solubility since the carbamates are systemic to roots via soil applications or through leaves from foliar applications [26].

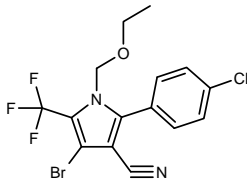
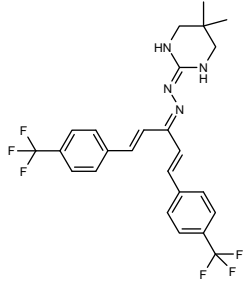
### 3. Environmental and Human Effects of Selected Chemicals Alternatives to Dicofol

Dicofol is a miticidal pesticide and acaricide, its precursor DDT was a revolutionary pesticide in agriculture, since it eradicated malaria from North America and Europe [27]. There is evidence that DDT plays a role in the aetiology such as pancreatic cancer, neuropsychological dysfunction, and reproductive outcomes. Research into these and other conditions would benefit from the same rigorous approaches used in breast cancer research. Until further high quality evidence is available, it is still too early, even 60 years after the introduction of this once ubiquitous chemical, to pass judgement on the role of DDT in a number of common diseases. This im-

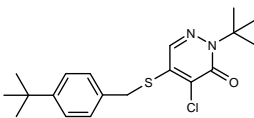
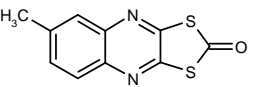
plies dicofol could produce the same injuries to humans since the structure similarities.

The possible alternatives listed show acaricide function as a majority function, **Table 2**, mode of action is for chlorfenapyr oxidative phosphorylation inhibition, given that it provokes a disruption of production of adenosine triphosphate (ATP) [29], this induces the cellular death and ultimately organism mortality, fenbutatin-oxide also inhibits oxidative phosphorylation [39], but this compound is non-systemic—a systemic pesticide is when the chemical is transported from the place of application to other parts of a plant or animal—so the product must usually be ingested by the organism [40], this usually affects beneficial insects. Dicofol has been widely use to control vine mites, the suggestion of DDT residues in dried fruit or wine products is potentially extremely damaging to viticultural industry. At this moment there are few chemical alternatives cheap and effective, as long as the only miticide presented here is oxythioquinox,

**Table 2. Summary of environmental and human effects of alternatives to dicofol.**

Compound	Description/ Way of action	Uses	Human adverse effects	Ref.	Associate impact	Ref.
 <p><b>CHLORFENAPYR</b></p> <p><b>Activity</b></p> <p>Acaricide, Insecticide</p>	<p>Halogenated pyrrol group. Its biological activity depends on its activation to another chemical compounds.</p> <p>Oxidative removal of the N-ethoxymethyl group of chlorfenapyr by mixed function oxidases forms an intermediate that uncouples oxidative phosphorylation at the mitochondria, resulting in disruption of production of ATP (adenosin triphosphate), cellular death and ultimately organism mortality.</p>	<p>Different crops as cotton, vegetables, citrus, top fruits, vines and Soya bean, ornamental crops.</p>	<p>Carcinogenic potential: Cannot be determined but suggestive.</p> <p>Tox Category II (acute test with the rat).</p> <p>Tox Category III (oral, dermal, and inhalation study results).</p> <p>No evidence of genotoxicity.</p>	<p>[28,29]</p>	<p>Due to its persistence and adverse reproductive effects in birds, it is banned in outdoor atmospheres; the green house use is not expected to result in outdoor residues, drift or runoff. So it is expected no wildlife exposure and other significant environmental exposure or risk since It is toxic to bees and high toxic to aquatic livings such as fish, prawn.</p>	<p>[29,30]</p>
 <p><b>HYDRAMETHYLNON</b></p> <p><b>Activity</b></p> <p>Insecticide, broad-spectrum fase effective acaricide.</p>	<p>Trifluoromethylamino hidrazones group, which act as a metabolic inhibitor.</p> <p>Causes death by inhibiting the formation of ATP (adenosine triphosphate); ATP provides the energy necessary for completing most biological processes, without ATP insects just die.</p>	<p>Control ants in grasses and rangelands and other non-crop lands (lawns, turf, and non-bearing nursery stock).</p> <p>Control of household ant species and cockroaches in non-food use areas in and around domestic dwellings and commercial establishments</p>	<p>Carcinogenic potential Group C: Possible Human Carcinogen (U.S. EPA, 1996).</p> <p>Tox Category III (acute test oral and dermal with the rat).</p> <p>Tox Category IV (acute test inhalation with the rat).</p> <p>No genotoxic in microbial test systems or clastogenic in cultured mammalian cells. Not induce dominant lethality in male rat germinal cells.</p>	<p>[28, 31, 32]</p>	<p>Especially toxic for fishes. High bioconcentration potential, even though its bioaccumulation potential is low. Moderated toxicity for mammals, birds, fishes an aquatic invertebrates, algae and beneficial organism.</p>	<p>[32,33]</p>

**Table 3. Summary of environmental and human effects of alternatives to dicofol.**

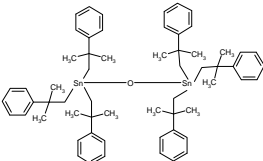
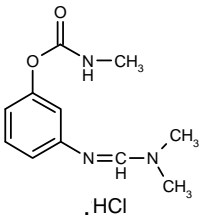
Compound	Description/ Way of action	Uses	Human adverse effects	Ref.	Associate impact	Ref.
 <p><b>PYRIDABEN</b></p>	<p>Pyridazone group.</p> <p>Acts as a mitochondrial electronic transport inhibitor at complex I mode of action.</p>	<p>Control of mite and white flies on ornamental plants, flowers and foliage (non-food) crops in green houses, and for the use to control mites on apples, pears and almonds. Grapes, apricots, cherries, nectarines, peaches, pistachio, plums, prunes and the tree nut group.</p>	<p>Carcinogenic potential: Group E: Evidence of Non-Carcinogenicity for humans based on the lack of evidence of carcinogenicity in male and female rats as well as in male and female mice.</p> <p>Toxicity to humans, including carcinogenicity, reproductive and developmental toxicity, neurotoxicity, and acute toxicity. Bioconcentration and bioaccumulation factors are low. It has been demonstrated human toxicity since it is harmful for neuroblastoma cells.</p>	[28,34]	<p>Water quality standards and physical properties affecting water contamination potential. Aquatic toxicity, bioconcentration and environmental fate of pyridaben are similar to synthetic pyrethroids used in agriculture, the main distinguishing feature is that pyridaben is more photo-labile than most pyrethroid, pyridaben can be photochemically degraded (Rand <i>et al.</i>, 2000) Acute-to-chronic ratios for pyridaben are low for fish and invertebrates indicating a low potential for residual activity.</p>	[35,36]
 <p><b>OXYTHIOQUINOX</b></p>	<p>Thiocarbamate group.</p> <p>The binding of oxythioquinox to proteins probable evolved a mechanism by which the sulfhydryl group of proteins initially attacked the carbonyl carbon of the acaricide, and the acaricidal action of oxythioquinox is due to the disruption of the normal function of significant proteins by the parent compound itself.</p>	<p>Only to use on non-food crops (landscape ornamentals) and places (nurseries and green-houses). It is reported to be incompatible with oils (causing phytotoxicity).</p>	<p>Carcinogenic potential: Group B2 (probable human carcinogen) based on lung tumours in males mice.</p> <p>Toxicity Category III or IV. Category II: Causes irreversible eye damage. Some bystanders may experience a skin reaction similar to sun burn, particularly if wind is present during applications.</p>	[28, 37,38]	<p>Moderately toxic to birds and adversely affected egg production, embryo survival (and perhaps fertility), hatch ability, offspring body weight and survival of offspring in avian reproduction studies.</p> <p>Highly toxic to fish and other aquatic organisms.</p>	[38]

**Table 3**, and it is no recommended for food crops, since it is a probable human carcinogen.

Formetanate hydrochloride has been applied predominantly to nectarines crops, and the way of action is similar to fenbutatin-oxide [41], **Table 4**, the toxicology is determined by its high solubility in water, more than 800.00 mg/l, since it is widespread in contaminated water, it supposes a great danger for aquatic organism, even though there is no complete data in the risk assessment about injuries to freshwater fish or invertebrates [42,43]. Its acetyl cholinesterase action can even affect to human, since it has acute toxicity via oral route. Formetanate

hydrochloride is considered a Group E, carcinogenic potential since there is evidence of no-carcinogenicity for humans [28]. Hydramethylnon is used to control ants and cockroaches in domestic and commercial establishments, but pyridaben is more used to control mites outdoor, in different crops of flowers and ornamental plants, and in grapes, apricots, etc. [31,32]. Pyridaben can be photochemically degraded, acute and chronic ratios for fish and invertebrates are low, as well as its bioconcentration and bioaccumulation potential, in front of dicofol. Hydramethylnon and pyridaben provoke death by inhibiting the formation of ATP [32,34], the difference is that

Table 4. Summary of environmental and human effects of alternatives to dicofol.

Compound	Description/ Way of action	Uses	Human adverse effects	Ref.	Associate impact	Ref.
 <p><b>FENBUTATIN-OXIDE</b></p> <p><b>Activity</b></p> <p>Acaricide.</p>	<p>Organotin compound.</p> <p>Selective, non systemic with contact and stomach action, acts by inhibiting oxidative phosphorylation at the site of dinitrophenol uncoupling, preventing the formation of the high-energy phosphate molecule adenosine triphosphate (ATP). Also inhibit photophosphorylation in chloroplasts, the chlorophyll-bearing subcellular units) and could therefore serve as algicide.</p>	<p>Citrus, blackberries and raspberries and grapes and pomes fruit, strawberries, cucumbers. Glasshouse crops.</p>	<p>Carcinogenic potential: Group E: Evidence of Non-Carcinogenicity for humans. Hazards to reproductions and development effects, not carcinogen.</p>	<p>[28,39]</p>	<p>Relative immobile and persistent in the environment, with no apparent major route of dissipation.</p> <p>Practically non-toxic to birds on an acute basis and extremely toxic to both freshwater and estuarine aquatic organisms.</p>	<p>[39,40]</p>
 <p><b>FORMETANATE HYDROCHLORIDE</b></p> <p><b>Activity</b></p> <p>Insecticide, acaricide.</p>	<p>Formamidine and carbamate groups.</p> <p>The toxic acts by contact and stomach action, by inhibiting acetylcholinesterase.</p>	<p>Alfalfa (grown for seed), apples, pears, peaches, nectarines and assorted citrus crops. There are no residential uses for this product.</p>	<p>Carcinogenic potential: Group E Evidence of Non-carcinogenicity for Humans. Known acetylcholinesterase inhibitor.</p>	<p>[28,41]</p>	<p>No indications of phytotoxicity on plants. No acute effects on threatened and endangered freshwater fish, invertebrates, and estuarine molluscs.</p>	<p>[42,43]</p>

hydramethylnon inhibits mitochondrial complex III electron transport (site II) and pyridaben inhibits mitochondrial complex I electron transport. It is known that this causes gradual degeneration of the dopamine neurons and reproduce many of the features of Parkinsonism. Pyridaben is a Group E, evidence of no-carcinogenicity for humans, and hydramethylnon and dicofol are classified as Group C, carcinogenic potential of possible human carcinogen [28].

#### 4. Conclusions

On the basis of available scientific data, different alternatives for substitution of dicofol examined here are directly related with the chemical structure, and the toxicity of these compounds is at least as potent as dicofol, affecting environmental and human beings.

Properties that identify substances as substances of high concern (CMR and PBT profile) are discussed in order to assess the possible substitution alternatives to dicofol. The understanding of the structure of compounds and their correlation to target organism is a very impor-

tant parameter to develop better designed pesticidal compounds with tailored toxicological properties on different pests. Mode of action of fluoroalkenyl derivatives is demonstrated to act in "specific manner" depending on the crop and the pest. Chemical inhibitor agents of mitochondrial electron transport are as dangerous as dicofol to environment and/or humans. The third group is better for humans but in most cases is worst for the environment, aquatic life specific. Common sense leads to make insecticide selection decisions which can ensure the most effective, least expensive and least environmentally disruptive methods, considering the mode of action, resistance, phytotoxicity, and the possibility of introduce other biological species to protect crops against pests.

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# Remote Mapping of Thermodynamic Index of Ecosystem Health Disturbance

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## ABSTRACT

*The study of the ecological system (ES) reaction to anthropogenous loading (AL) has been aimed at developing the remote sensing method for quantitative mapping of AL on ES. The analysis of the problem has shown that the main approach for its solution is to assess the amount of entropy induced in ES by AL. The general formalism has been discussed and the thermodynamic index of ES health disturbance (TIEHD- $I_T$ ) has been deduced from the conservation law as a portion of solar exergy spent by ES on the parrying entropy formed in ES due to AL with respect to the total amount of exergy of solar irradiations absorbed by ES. The technique of remote mapping of TIEHD has been developed. The maps of TIEHD and the normalized differential vegetation index (NDVI)- $I_V$  have been compiled on the basis of NOAA and EOS satellite data. The qualitative and quantitative analysis exhibited the best sensitivity of TIEHD to AL on ES in respect to NDVI.*

**Keywords:** Ecosystem, Thermodynamics, Anthropogenous Loading, Satellite, Index

## 1. Introduction

Quantitative estimation of ES health affected by AL is one of the most challenging problems of environmental state monitoring. In terms of economy remote sensing technology is the most convenient for carrying out operational monitoring of ES reaction to AL.

There have been numerous attempts to create techniques for assessing AL on ES [1,2]. These techniques are mainly based on the method of induction (from the particular to the general) using numerous quantitative indicators, e.g., indices, describing [1]:

- 1) the biogeochemical substance and energy cycles;
- 2) the actual or potential productivity of the ES;
- 3) the biodiversity of the ecosystems (Species Richness, ES Scarcity, ES Vulnerability);
- 4) the cultural value of the affected sites;
- 5) the migration and dispersal of species composition.

W. M. Achten, E. Mathijias, B. Muys [3] based their ES health criteria system on two factors: impact on the ES Structural Quality (Soil fertility, Biomass production, Species diversity) and ES Functional Quality (Soil structure, Vegetation structure, On-site water balance). The authors suggested 22 indicators of AL on ES and the principle of indicators generalization: "...the impact in-

dicator scores as the summation of the relative impacts of the different land use activities" [3].

Our analysis of the above mentioned factors leads to the conclusion that this methodology of assessment of AL on ES cannot be implemented in a real operational mode due to its high complexity and for economic reasons.

There are two different approaches to the solution of scientific problems: microscopic and macroscopic. Statistical thermodynamics, for example, uses the microscopic approach, first, to derive parameters of kinematics and dynamics for each particle of ideal gas, then, to describe the type of chaos, further, to take into account the type of interactions between particles and, finally, to obtain some integrated parameters of the investigated object, e.g. a spatial temperature distribution in the ideal gas. However, the same result (temperature distribution in a solid body) can be more easily obtained using the macroscopic approach. In this case some general parameters of the heat transfer in a solid body (a thermal conductivity, a thermal capacity and a density) are to be measured, followed by a boundary value problem solution of heat transfer using Fourier differential equation. This approach needs much less effort to obtain the same result than the first case.

The results in [1,3] have been obtained using the microscopic approach (collecting a large number of different indicators for each ES element and compiling a generalized index). Therefore it was found reasonable to make an attempt to create a generalized index of AL on ES using the macroscopic approach. These attempts were made in the framework of the ES thermodynamic theory [4-9].

The main idea of the thermodynamic approach of ES health assessment is to reveal changes of generalized ES thermodynamic parameters caused by AL. Especially noteworthy is the paper concerned mainly with philosophical problems of the thermodynamics of biological organisms by E. D. Schnider and J. J. Kay [5] who formulated nine principles of thermodynamic state of ES. T. Wagendorp *et al.* reduced these principles to two basic ones (these methods of assessment of ES reaction to AL are discussed in [9]). Their technique includes a description of the structural state of the ES (goal function: maximum exergy<sup>1</sup> storage) and the function caused by the low entropy system (goal function: maximum exergy dissipation). According to T. Wagendorp *et al.* only two indices can be used for remote mapping: thermal response number ( $I_{TRN}$ ) and solar exergy dissipation ( $I_{SED}$ ), put forward by J. C. Luvall and H. R. Holbo [7] as:

$$I_{TRN} = \sum_{\tau_1}^{\tau_2} [(R_n \times \Delta\tau) / \Delta T_s] \quad (1)$$

where  $R_n$  is the net incoming radiation;  $\Delta\tau = (\tau_2 - \tau_1)$  is the time interval between two successive remote sensing surveys;  $\Delta T_s$  is the change of surface temperature  $T_s$  between moments of time  $\tau_1$  and  $\tau_2$ .

$$I_{SED} = R_n / K^* \quad (2)$$

where  $K^*$  is the net shortwave radiation.

$I_{SED}$  represents the fraction of the net radiation that is dissipated into a heat – the type of energy with a lower level of exergy. It reflects an exergy degradation and storage in a system [5]. J. C. Luvall and H. R. Holbo mapped  $I_{TRN}$  and  $I_{SED}$  applying airborne multispectral survey with the help of a Thermal Infrared Multichannel Scanner (TIMS) [7].

The analysis of these two indices has shown the following deficiencies:

- 1) these two indices have not been deduced from the basic physical laws, but were suggested empirically;
- 2)  $I_{TRN}$  is the palliative of the thermal inertia (TI) (see [10-14] for details).

The TI reflects the resistance of the surface to the periodic process of heating and cooling. Being a bulk prop-

erty, TI does not depend on the time of observation and weather conditions.  $I_{TRN}$ , being just a characteristics, depends on different natural factors. That is why it is impossible to quantitatively compare  $I_{TRN}$  obtained on different dates, even for the same ES.

The best results in the macroscopic approach have been achieved by S. E. Jorgensen and Yu. Svirezhev in their monograph on the general thermodynamic theory of ES [4]. The authors demonstrated that the general thermodynamic measure of AL on ES is the additional production of entropy  $\sigma_\tau$  taking place in the time interval  $\Delta\tau$  [4]:

$$\sigma_\tau = [S(\tau_o + \tau) - S(\tau_o)] = [\hat{W}_f + \hat{W}_{ch} + \langle P \rangle \Delta\tau - P_o \Delta\tau] / T; \quad (3)$$

where:  $S$  is the entropy of ES;  $\hat{W}_f$  is the total energy loading on ES;  $\hat{W}_{ch}$  is the total chemical loading on ES;  $P$  is the gross primary production measured in mass of carbon per unit area per year: ( $g \text{ Carbon}/m^2/yr$ ). The gross primary production is the speed at which an ES stores a given amount of chemical energy as a biomass in a given time interval.  $\langle P \rangle$  is the mean gross primary production averaged over the  $\Delta\tau$  time of AL;  $P_o$  is the initial gross primary production of ES for the moment of time:  $\tau_o$ , when AL was applied;  $T$  is the absolute temperature of ES (assumed constant during all time interval  $\Delta\tau$ );  $\Delta\tau = \tau - \tau_o$ ;  $\tau$  is the ongoing moment of time.

The analysis of Equation (3) shows that remote measurements of  $\hat{W}_f, \langle P \rangle, P_o, T$  are possible to make. However, to determine remotely, the chemical loading ( $\hat{W}_{ch}$ ), is very difficult due to a large number of uncertainties making it impossible to use Equation (3) directly. Thus, the main target of this paper is to develop TIEHD that can be mapped remotely, based on general thermodynamic considerations.

The outline of the paper is as follows: Section 2 presents our formalism and derives a general formula for TIEHD. Section 3 deals with materials and methods. Section 4 shows the results for the industrial Urals region, one of the most industrialized and polluted areas of Russia. Discussion and conclusions are presented in Section 5 and Section 6.

## 2. General Formalism

Following the major works concerned with the thermodynamic theory of ES [4,5,9] we suggest that ES and the environment is a supersystem and the environment is much larger than ES. According to Equation (3) AL

<sup>1</sup>Ex-exergy is the maximum work, which ES can perform during the process of reaching the state of equilibrium with the environment [4].



manifests itself as an increase in ES entropy. This is the key factor of the thermodynamic approach to AL assessment. Before discussing the possible techniques of remote mapping of AL on ES it is necessary to additionally emphasize some very important points of the ES thermodynamic theory.

Reference [4] emphasizes the following: “*Loss of exergy and production of entropy are two different descriptions of the same reality*”. The important concept of the “entropy pump” was expressed by Yu. M. Svirezhev [4]. The concept of the “entropy pump” assumes that it removes entropy, which normally generates in ES by expending the absorbed exergy of solar radiation. As a result, ES does not accumulate the entropy [4].

To find an indirect assessment of ES degradation under AL one should consider the steady state equilibrium in ES in accordance with [4], *i.e.* the annual entropy balance of ES is equal to zero<sup>2</sup>. It was shown in [4] that under these assumptions the following equation can be written:

$$P_0 - \langle P \rangle = (\hat{W}_f + \hat{W}_{ch}) / \Delta \tau, \quad (4)$$

The important conclusion resulting from (4) is as follows: *the productivity of ES changes due to AL*. To put it in other words, ES can decrease (or increase in case of fertilization) its biomass productivity, depending on the amount and type of AL. It means that the solar exergy input to ES is spent on:

- 1) the standard entropy removing from ES (in other case a degradation of biomass of ES must take place);
- 2) removing of the additional entropy induced by AL;

As a result of AL, the productivity of ES decreases. If the productivity of the ES biomass under AL reaches zero, the further AL increase will result in the degradation of ES biomass.

Taking into account the above mentioned considerations, the exergy balance of ES is as follows:

$$\hat{E}x_o = \hat{E}x_c + \hat{E}x_e + \hat{E}x_a \quad (5)$$

where  $\hat{E}x_o$  is the specific flux density of the exergy of solar irradiation assimilated by ES,  $W/m^2$ ;  $\hat{E}x_c$  is the portion of  $\hat{E}x_o$ , spent by ES on carbon deposition in ES (a biomass production),  $W/m^2$ ;  $\hat{E}x_e$  is the portion of  $\hat{E}x_o$ , spent by ES on standard entropy removing from ES,  $W/m^2$ ;  $\hat{E}x_a$  is the portion of  $\hat{E}x_o$ , spent by ES on the

parrying of AL,  $W/m^2$ .

Equation (5) shows that solar exergy is spent by ES on a number of processes, such as removing the entropy of biomass (an “entropy pump”), carbon deposition into biomass, AL parrying.

Following [4,9], let's assume the reference ES (background ES), *i.e.*, ES which has not been affected by AL (actually, such ES's are specially preserved natural areas). Then, for the reference (background) ES it follows from Equation (5) that:

$$\hat{E}x_o^b = \hat{E}x_c^b + \hat{E}x_e^b \quad (6)$$

where index “b” indicates the background ES.

$\hat{E}x_a$  may be expressed after subtracting Equation (5) from Equation (6) as:

$$\hat{E}x_a = \Delta \hat{E}x_c + \Delta \hat{E}x_e - \Delta \hat{E}x_o \quad (7)$$

where:

$$\Delta \hat{E}x_o = \hat{E}x_o^b - \hat{E}x_o;$$

$$\Delta \hat{E}x_c = \hat{E}x_c^b - \hat{E}x_c;$$

$$\Delta \hat{E}x_e = \hat{E}x_e^b - \hat{E}x_e;$$

Taking into account that in this case ES is not shifted far from the equilibrium state we can assume that  $\Delta \hat{E}x_o$  and  $\Delta \hat{E}x_e$  are quantities which are much smaller than the other members of Equation (7). Moreover, it is suggested that AL on ES is parrd by ES, spending the exergy normally used for carbon deposition. Thus, it is the key moment to assign TIEHD, which leads to the conclusion that when AL increases,  $\Delta \hat{E}x_c$  increases accordingly. That is why  $\Delta \hat{E}x_c$  can be used as the indicator of AL.

TIEHD-  $I_T$  can be written as:

$$I_T = Ex_a / \hat{E}x_o \approx \Delta \hat{E}x_c / \hat{E}x_o \quad (8)$$

According to Equation (8) TIEHD is the portion ( $\hat{E}x_a$ ) of the solar exergy absorbed by ES ( $\hat{E}x_o$ ), spent on the parrying of AL. The right side of Equation (8) makes it possible to calculate TIEHD on the basis of remote (airborne or satellite flown) measurements.

According to Equation (8), for the background ES  $I_T = 0$ , because this ES was not affected by AL ( $\hat{E}x_a = 0$ ). In case ES is affected by AL:  $1 > I_T > 0$ . Thus,  $I_T$  reflects the level of ES health disturbance after AL<sup>3</sup>.

Concluding this section it is necessary to emphasize the following advantages of TIEHD:

- 1) unlike the above mentioned indices (see Equations (1) and (2)), which were suggested empirically, TIEHD is derived on the basis of the conservation principle (see Equation (5));

<sup>2</sup>It means that ES is not shifted too far from the equilibrium state as a result of AL, and the amount of solar exergy assimilated by ES is enough to remove all entropy produced by AL from ES.

<sup>3</sup>In reality, besides AL disturbing ES, different natural events (for example, forest and grass fires, a mass breeding of different insects, etc. can disturb ES as well.

2) it can be suggested that TIEHD is more sensitive to AL than remotely measured vegetation indices, as TIEHD reflects the changes in the physiological process of vegetation (transpiration and photosynthesis).

Therefore, the remote mapping of the rate of carbon deposition and  $\hat{E}x_o$  provides the possibility to compile a map of TIEHD.

### 3. Materials and Methods

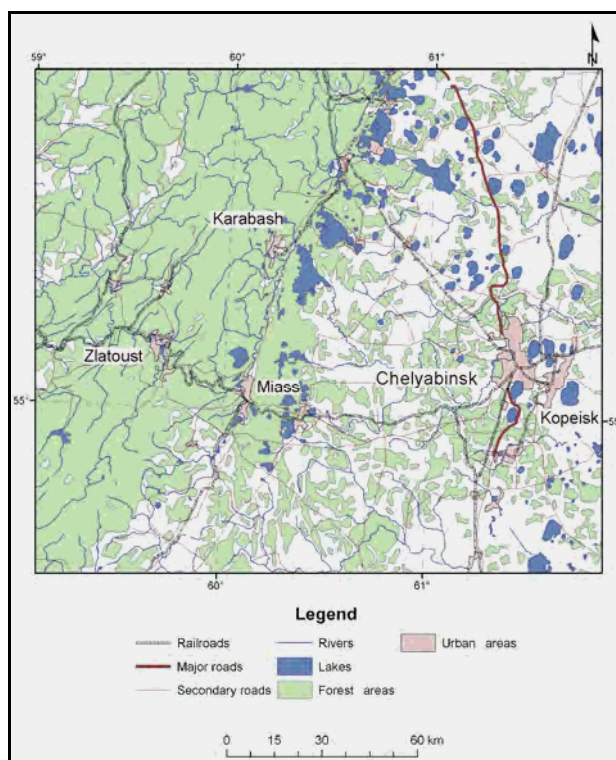
#### 3.1. Test Site

The south-eastern Urals region of Russia has been chosen as the test site (the Urals Test Site (UTS)) for TIEHD remote mapping (**Figure 1**).

The climate of UTS is continental with long cold winters and relatively short and warm summers [15]. There are three biomes within the UTS territory: boreal forest (UTS Western part), South of West Siberian forest steppe, and steppe (UTS Eastern part) [15].

There are two major geomorphologic forms within the UTS territory: Trans-Urals peneplain (UTS Western part) and West Siberian lowland (Eastern part).

The UTS territory lies in one of Russia's oldest industrial regions with a large number of metallurgical, metalworking, engineering, and chemical enterprises. The biggest industrial centers are the cities of Chelyabinsk, Karabash, Kopeisk, Miass, Zlatoust (see **Figure 1**).



**Figure 1.** The schematic map of the UTS.

The main requirements for TIEHD remote mapping technique verification were the following: the total range of AL on ES has to be varied from maximum to minimum; the test site ES has to be homogeneous enough and the AL source has to be unique.

The city of Karabash in Chelyabinsk Oblast' is the one of such places within UTS (**Figure 1**).

The city of Karabash is located in a mountainous taiga area. The main species of the forests are spruce, silver fir and an admixture of pines, mountain ashes and junipers. The soil is mountainous podzol. The main wind direction is latitudinal. The city is situated in the depression of the surface relief.

The copper smelting plant was built in the city of Karabash early in the 19th century. Besides, the smelting of complex sulfide ores started at the beginning of the 20th century. As a result, the surrounding ES was significantly affected by this enterprise at the end of the 20th century. For example, during the peak of the Karabash enterprise production (till 1989) about 160-180 thousand tons of pollutants in the form of smoke, dust and gases were thrown out into the atmosphere annually. About 90% of it was sulfur dioxide, the others being lead, copper, zinc, arsenic, as well as carbon oxide, nitrogen dioxide, etc. 12 million tons of harmful substances have been thrown out into the atmosphere during all the time of the industrial complex activity. As a result, ES around the city of Karabash has been affected by the hard AL during a long time period. This is illustrated in the map of NDVI- $I_V$  (see **Figure 5**). The broad area of dead vegetation can be observed inside and around the city of Karabash.

#### 3.2. Data

Terra(MODIS) satellite data were used to calculate  $\hat{E}x_o$  (Equation (9), **Table 1**).

#### 3.3. $\hat{E}x_o$ Mapping

The  $\hat{E}x_o$  can be measured remotely using the technique described by S. Yorgensen and Yu. Svirezhev [4]:

$$\hat{E}x_o = \int_{\Omega} Q^{out}(\nu) \ln[Q^{out}(\nu) / Q^{in}(\nu)] d\nu + R = R[1 + \bar{\alpha}(Ku + \ln \bar{\alpha} - 1) / (1 - \bar{\alpha})]; \quad (9)$$

where  $Q^{out}$ ,  $Q^{in}$  are the outgoing and the ingoing specific densities of solar irradiation fluxes respectively,  $W/m^2$ ;  $\nu$  is the frequency of light spectrum;  $\Omega$  is the total measured spectral interval;  $K = \sum_{i=1}^n p_i \log(p_i / p_i^0)$

is Kullback's measure;  $p_i^0$ ,  $p_i$  probabilities before and

**Table 1. Satellite data, used for the map of TIEHD compilation.**

Date	Time GMT, hrs:min:s	Satellite	Instrument	Channels
June, 4, 2009	07:00:00	NOAA-18	AVHRR	4,5
	07:00:00	Terra	MODIS	1-7,31,32
	08:50:00	Aqua	MODIS	1-7,31,32
	15:23:00	NOAA-17	AVHRR	4,5
	18:15:00	Terra	MODIS	31,32
	22:00:00	Aqua	MODIS	31,32
June, 5, 2009	01:31:00	NOAA-15	AVHRR	4,5
	05:35:00	NOAA-17	AVHRR	4,5
	07:45:00	Terra	MODIS	1,2,31,32
	07:55:00	Aqua	MODIS	1,2,31,32
	21:25:00	Aqua	MODIS	31,32

after the interaction respectively;  $R$  is the radiation balance at ES surface,  $W/m^2$ ;  $\bar{\alpha} = Q^{out} / Q^{in}$  is the surface albedo.

For the Terra(MODIS) satellite data, the surface albedo can be mapped by using the standard MODIS Level 1B product, described in [16].

The result of mapping of  $\hat{E}x_o$  inside the UTS has been shown in **Figure 2**.

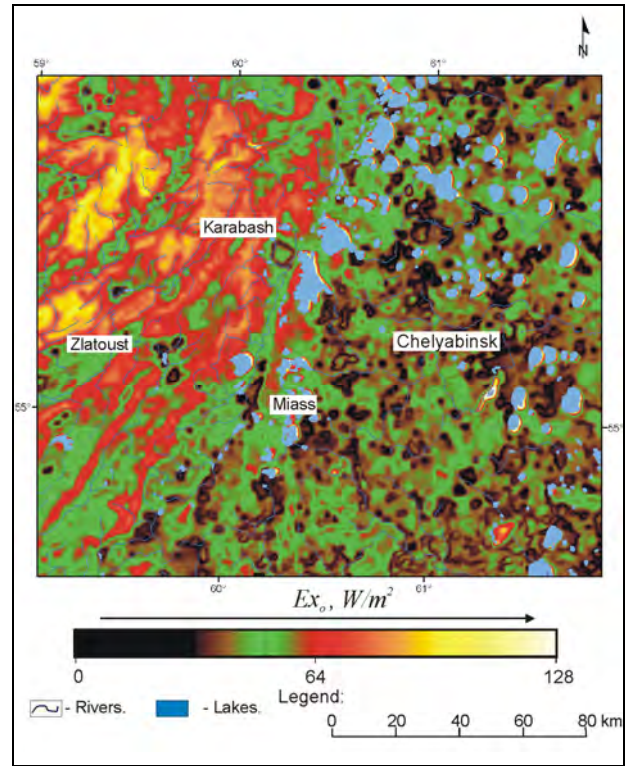
### 3.4. Mapping of Carbon Deposition in ES

The portion of  $\hat{E}x_o$  spent by ES on carbon deposition  $\hat{E}x_c$  directly depends on the amount of water evaporated by ES [4]. It is necessary for vegetation to dissipate 278 KJ of heat for 1 g of carbon deposition as a biomass [4]. Hence, the following formula can be presented as:

$$\hat{E}x_c = a \times b \times \bar{E} = 3.66 \times \bar{E} \quad (10)$$

where:  $\bar{E}$  is the specific daily mean average evaporation rate,  $m^3 / (m^2 \cdot s)$ ;  $a = 1/879$ , (kg of carbon/ $m^3$  of  $H_2O$ ), the carbon/water factor (ES has to evaporate 879  $m^3$  of water to assimilate 1 kg of carbon);  $b$  is the factor of specific energy of carbon assimilation ( $278 \cdot 10^6 J / (kg \text{ of carbon})$ ).

The method used for  $\bar{E}$  remote mapping is based on TI approach [11-14,17-20] (**Figure 4**). This approach uses the daily variation of the land surface temperature  $T_s$  as a mathematical model, taking into account the basic factors affecting  $T_s$  formation. Our model [11] assumes the following: the meteorological conditions and concentration of optically active gases in the atmosphere within



**Figure 2. The map of  $\hat{E}x_o$ , compiled for the territory of UTS on the basis of Terra(MODIS) satellite data.**

the whole study area are identical; emissivity, albedo of land surface, and TI do not vary during the whole period of the satellite observations. To determine  $\bar{E}$ , we use the results of a thermal & multispectral satellite survey conducted for several days under stable meteorological conditions in the absence of rainfall with the aim to characterize the daily  $T_s$  dynamics more precisely. Moreover,  $\bar{E}$  mapping is based on the following parameters of routine meteorological observations, involved in the mathematical model of  $T_s$ :

- 1) total solar radiation,
- 2) air temperature, air moisture, and wind velocity at a height of 2 m above the surface,
- 3) atmospheric pressure,
- 4) cloudiness.

To solve the inverse problem the look-up table method was used, following J. C. Price [18]. Mathematical simulations of  $T_s$  were performed for all possible combinations of TI,  $\bar{E}$ , a heat flux and an albedo, i.e., the “library” of  $T_s$ . Then the measured  $T_s$  values were compared with the simulated quantities. The values sought  $\bar{E}$  were deduced in accordance with the assigned fitting criteria of the measured and simulated  $T_s$  values. When this algorithm is used, the systematic error of the inverse problem solution for  $\bar{E}$  (**Figure 4**) equals 0.3 mm/



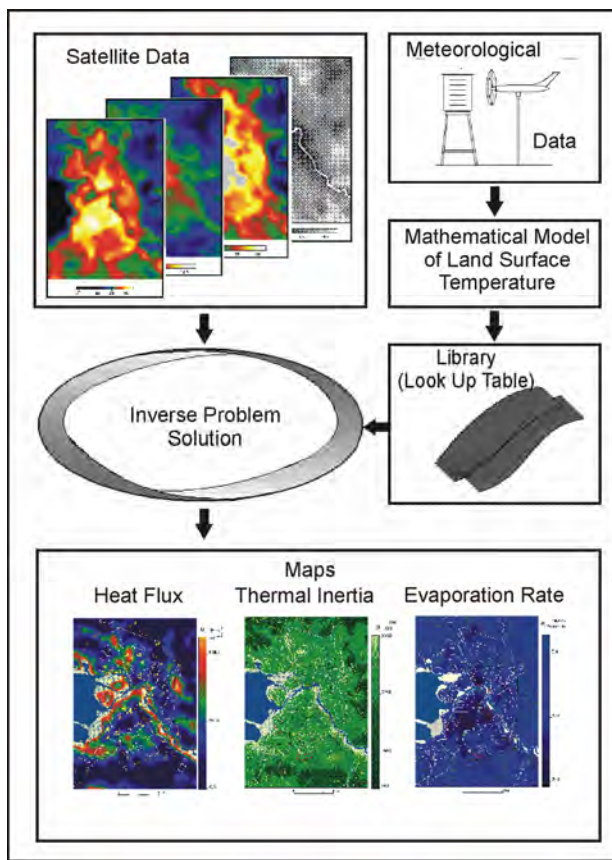


Figure 3. The algorithm of TI,  $\bar{E}$  and the heat flux mapping on the basis of multiple satellite survey and meteorological observation data.

$(m^2 \cdot day)$ , while the root-mean square error equals  $0.1 \text{ mm} / (m^2 \cdot day)$ .

The analysis of  $\bar{E}$  spatial distribution (Figure 5) shows the above described differences in ES between Western and Eastern parts of UTS. In the framework of the Western part one can observe more intensive  $\bar{E}$ , than those of the Eastern part. After that  $\hat{E}x_c$  has been calculated according to Equation (8).

Finally, it should be stressed that the TI-approach based algorithm was used because it allowed the remote mapping of the daily mean averaged evaporation rate -  $\bar{E}$ . However, by now the maximum spatial resolution of satellite scanners that enable multiple daily surveys in the infrared-thermal spectral band has not been more than 1 km. That is why TI approach can't be used for more detailed mapping with the help of, e.g. Landsat TM (ETM+) or ASTER infrared-thermal & multispectral images. In this case the methods described in [6,19,20] are preferable. However, the evaporation rate obtained by using single survey methods is not daily mean averaged and reflects the moment of time during which the satellite

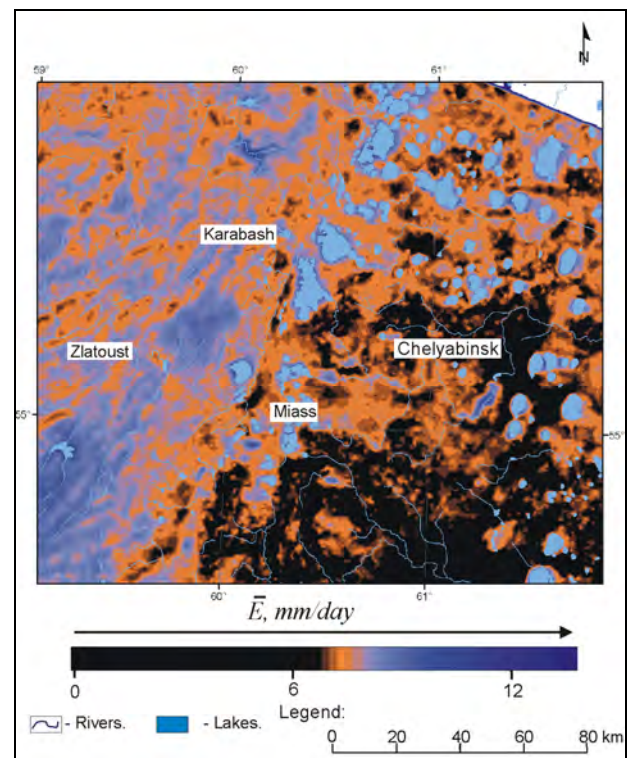


Figure 4. The map of  $\bar{E}$ , compiled by using the Terra(MODIS) and NOAA(AVHRR) satellite data.

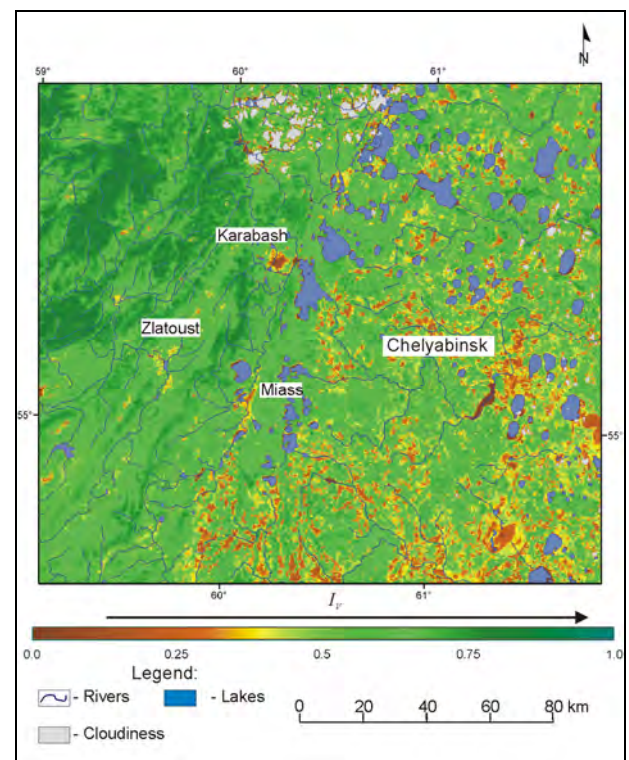


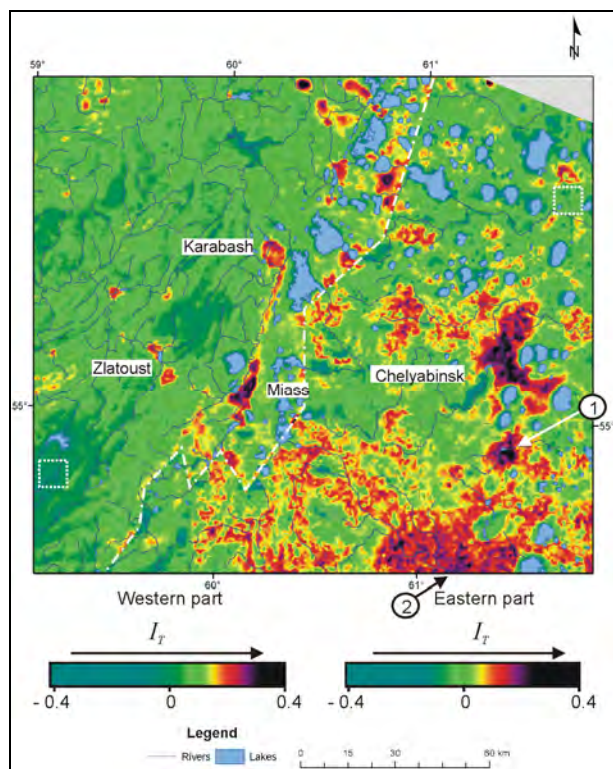
Figure 5. The map of NDVI for the UTS, according the Terra (MODIS) satellite survey on June 4, 2009.

survey has been done.

As it follows from the Equation (8), if one knows the spatial distributions of  $\hat{E}x_c$ , as well as,  $\hat{E}x_o$ , the  $I_T$  can be calculated.

#### 4. Results

Due to the big difference between ESs of the Western and Eastern parts of UTS the TIEHD calculation was carried out separately for each part according to the right part of Equation (8). The result is shown in **Figure 6**. The boundary between the Western and Eastern parts is marked by the white stroke-dashed line. This boundary was plotted on the basis of the visual interpretation of satellite data. For the best accuracy it should be plotted with the help of unsupervised classification of satellite data. Two areas of background ES's have been selected for each part of the UTS (both Western and Eastern) (shown by white dotted lines in **Figure 6**) as the areas of maximal  $\bar{E}$ . Calculations of TIEHD were made for each part of the UTS separately according to the background ES (unaffected by AL).



**Figure 6.** The map of TIEHD compiled for the UTS on the basis of satellite data. The boundary between the Western and Eastern ESs is marked by the white stroke-dashed line. The white dotted line marks background ESs for Western and Eastern parts of UTS. 1. The Korkino Open Cast for brown coal mining; 2. The location of Yuzhno-Ural'skaya and Troitskaya Electricity Power Plants.

#### 5. Discussion

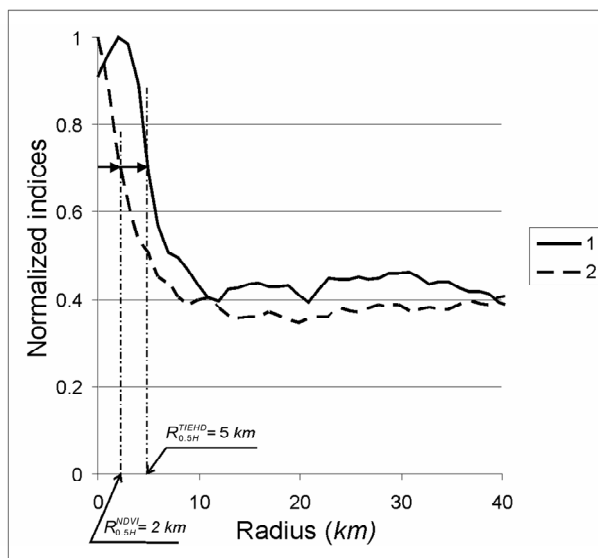
The visual analysis of the set of maps (**Figure 6**), (**Figure 1**) and (**Figure 5**) leads to the following conclusions:

1) the urban and industrial areas are characterized by a high level of TIEHD;

2) the areas of high TIEHD, which indicate urban and industrial areas, are bigger than the areas of low NDVI, indicating the same sources of AL.

Besides the visual analysis, the comparison of TIEHD and NDVI sensitivity to AL on ES's for the territory around the city of Karabash was made quantitatively (for the Western part of the UTS only). The water surfaces of lakes, as well as clouds were masked on the TIEHD and NDVI maps. After that, the normalized indices were calculated and plotted in **Figure 7** as Normalized TIEHD:  $(I_T)/(I_T)_{\max}$  and Normalized Complementary NDVI:  $(1-I_V)/(1-I_V)_{\max}$ . These indices have been averaged inside the Western semi-circumference for each pixel of the radius, plotted from the center of the city of Karabash. As a result, **Figure 7** indicates that the Normalized TIEHD varies from  $I_T = 1.0$  at the radius of  $R \approx 2$  km from the Karabash city center up to  $I_T \approx 0.4$  for the radius of  $R = 11$  km from that center. At the same time the Normalized Complementary NDVI has maximum ( $I_V = 1.0$ ) exactly at the center of the city of Karabash and the minimum ( $I_V = 0.36$ ) at the radius of  $R \approx 12$  km.

Let's assume that the width of the indice's peak at the



**Figure 7.** Comparison of Normalized TIEHD and the Normalized Complementary NDVI sensitivity to the AL on ES around the city of Karabash, the UTS. 1. Normalized TIEHD:  $(I_T)/(I_T)_{\max}$ ; 2. Normalized Complementary NDVI:  $(1-I_V)/(1-I_V)_{\max}$ .

half of the peak's height ( $2R_{0.5H}$ ) indicates the sensitivity of indices to AL. In this case, for the city of Karabash, according the Normalized TIEHD the  $2R_{0.5}^{TIEHD} = 10 \text{ km}$  (**Figure 7**), while for the Normalized Complementary NDVI such width  $2R_{0.5H}^{NDVI} = 4 \text{ km}$  (**Figure 7**). This leads to the conclusion that the sensitivity of TIEHD to AL is 2.5 times better, compared to NDVI.

The highest sensitivity of TIEHD to AL on ES can be explained by the following considerations. TIEHD is calculated using such characteristics of vegetation physiological processes as photosynthesis activity and quality, as well as water transpiration by leaves. However, the vegetation index reflects the presence of chlorophyll in ES. That is why, it can be suggested that the vegetation index records the dramatic stage of vegetation degradation, when the vegetation has lost chlorophyll (resulting from forest cuttings, forest and steppe fires or vegetation death after acid industrial precipitations, as in the case of the city of Karabash, etc.).

The comparison between TIEHD inside the Western part of UTS (**Figure 6**) and TIEHD inside the Eastern part of UTS shows a big difference, with the Eastern part having the highest TIEHD. It can be explained by the landscape difference of these two parts, as well as by different anthropogenic activity. As can be seen in **Figure 2**, the Western part of the UTS is covered by forest, while the Eastern part is mainly covered by cultivated fields. The map of TIEHD was compiled for the beginning of summer. For the agriculture & climatic zone of UTS it is the time with no vegetation on the surface of fallow fields. As a result, there are areas of the bare soil here, whose evaporation rate is much lower than that of the forested area. Thus, TIEHD of the cultivated area for the beginning of June is much bigger than TIEHD of the forested area which is not affected by AL.

The big urban and industrial area around the city of Chelyabinsk is characterized by high TIEHD, resulting from very big AL on ES. Two more areas of high TIEHD were investigated additionally. For example, the area of high TIEHD, situated to the south of the city of Chelyabinsk (1 in **Figure 6**) indicates the giant Korkino Open Cast. Its depth reaches 500 m. The Open Cast supplies Yuzhno-Ural'skaya and Troitskaya Electric Power Plants with brown coal. The total power of the Electric Power Plants is ~ 3000 MW. One of the possible reasons for a very big area of high TIEHD at the Southern border of UTS (2 in **Figure 6**) is the pollution of ES resulting from the smoke of these Electric Power Plants.

Unlike this situation, water basins (lakes, water ponds, etc) are surrounded by the zones of very low TIEHD (**Figure 6**), as, according to Russia's legislation the zones surrounding water basins are territories specially

protected from AL. It is one of the explanations of the above mentioned phenomenon.

## 6. Conclusions

To summarize, the major regularities of thermodynamic reply of ES on AL have been presented and discussed. Based on the conservation law of exergy fluxes inside ES, it has been shown that after some simple assumptions TIEHD can be represented as a portion of solar exergy spent by ES on the parrying entropy formed in ES due to AL with respect to the total amount of exergy of solar irradiations absorbed by ES. It has also been shown that TIEHD may be mapped on the basis of remote (airborne or satellite flown) measurements made in the visible, nearest infrared and infrared-thermal spectral bands by optic & electronic scanners.

We have chosen the Southern-Eastern Ural region of Russia as the Test Site and compiled the map of TIEHD by using NOAA and EOS satellite data. The preliminary analysis exhibits a better sensitivity of TIEHD to AL on ES than the NDVI.

It can be concluded that the macroscopic approach (it was used for the TIEHD compilation) is simpler and less expensive for the satellite monitoring of AL on ES than the microscopic approach based on big a number of different indices and their generalization.

The further directions extending our research results include, in particular, the investigation of TIEHD seasonal dynamic and the detailed ground truth of TIEHD maps.

## 7. Acknowledgements

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# Earthworms: Charles Darwin's 'Unheralded Soldiers of Mankind': Protective & Productive for Man & Environment

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## ABSTRACT

*Earthworms promises to provide cheaper solutions to several social, economic and environmental problems plaguing the human society. Earthworms can safely manage all municipal and industrial organic wastes including sewage sludge and divert them from ending up in the landfills. Their body work as a 'biofilter' and they can 'purify' and also 'disinfect' and 'detoxify' municipal and several industrial wastewater. They reduce the BOD & COD loads and the TDSS of wastewater significantly. They can even remove the EDCs (endocrine disrupting chemicals) from sewage which is not removed by the conventional sewage treatments plants. Earthworms can bio-accumulate and bio-transform many chemical contaminants including heavy metals and organic pollutants in soil and clean-up the contaminated lands for re-development. Earthworms restore & improve soil fertility by their secretions (growth hormones) and excreta (vermicast with beneficial soil microbes) & boost 'crop productivity'. They have potential to replace the environmentally destructive chemical fertilizers from farm production. The 'protein rich' earthworm biomass is being used for production of 'nutritive feed materials' for fishery, dairy & poultry industries. They are also being used as 'raw materials' for rubber, lubricant and detergent industries. The bioactive compounds isolated from earthworms are finding new uses in production of 'life saving medicines' for cardiovascular diseases and cancer cure.*

**Keywords:** Detoxifying, Disinfecting, Waste Degradation, Wastewater Purification, Soil Decontamination, Soil Fertility, Crop Production, Earthworms Medicines, Nutritive Feed

## 1. Introduction

A revolution is unfolding in vermiculture studies for multiple uses in environmental protection and sustainable development. Earthworms have over 600 million years of experience as 'environmental managers' in the ecosystem. Vermiculture scientists all over the world knew about the role of earthworms as 'waste managers', as 'soil managers & fertility improvers' and 'plant growth promoters' for long time. But some comparatively 'new discoveries' about their role in 'treatment of municipal and industrial wastewaters', 'remediation of chemically contaminated soils' and 'development of life saving medicines', 'nutritive feed materials' for fishery & dairy industries and raw materials for 'rubber, lubricants, soaps & detergent industries' have revolutionized the studies into vermiculture.

Earthworms promises to provide cheaper solutions to

several social, economic and environmental problems of human society. They are both 'protective' & 'productive' for environment and society. They protect the environment (by remedifying the contaminated soil, degrading the solid wastes and purifying wastewater) and also produce nutritive 'protein rich feed materials' for cattle and 'organic fertilizers' for the farmers to grow safe and chemical-free organic foods for society [1].

## 2. The Biology & Ecology of Earthworms

Earthworms are long, narrow, cylindrical, bilaterally symmetrical, segmented animals without bones. Usually the life span of an earthworm is about 3 to 7 years depending upon the type of species and the ecological situation. Earthworms harbor millions of 'nitrogen-fixing' and 'decomposer microbes' in their gut. They have 'chemoreceptors' which aid in search of food. Their body con-

tains 65% protein (70-80% high quality 'lysine rich protein' on a dry weight basis), 14% fats, 14% carbohydrates and 3% ash [2-4].

Earthworms occur in diverse habitats specially those which are dark and moist. They can tolerate a temperature range between 5°C to 29°C. A temperature of 20°C to 25°C and a moisture of 60-75% is optimum for good worm function. Earthworms multiply very rapidly. Studies indicate that they double their number at least every 60-70 days. Given the optimal conditions of moisture, temperature and feeding materials earthworms can multiply by  $2^8$  i.e. 256 worms every 6 months from a single individual. Each of the 256 worms multiplies in the same proportion to produce a huge biomass of worms in a short time. The total life-cycle of the worms is about 220 days. They produce 300-400 young ones within this life period [5]. Earthworms continue to grow throughout their life.

### 3. Earthworms: The Protector of Human Environment

Earthworms are 'unheralded soldiers of mankind' created by Mother Nature. Although the great visionary scientist Sir Charles Darwin indicated about them long back but very few biologists really realized that. Now it is being realized and revived all over the world and services of earthworms are being utilized with a technological approach. Some of the virtues of earthworms given below

#### 3.1. Tremendous Abilities & Ecological Adaptation for Survival in Harsh Environment

Earthworms can tolerate toxic chemicals in environment. After the Seveso chemical plant explosion in 1976 in Italy, when vast inhabited area was contaminated with certain chemicals including the extremely toxic TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin) several fauna perished but for the earthworms that were alone able to survive [6].

#### 3.2. Tolerate and Bio-Accumulate Toxic Soil Chemicals & Contaminants from Environment

Several studies have found that earthworms effectively bio-accumulate or biodegrade several organic and inorganic chemicals including 'heavy metals', 'organochlorine pesticide' and micropollutants like 'polycyclic aromatic hydrocarbons' (PAHs) residues in the medium in which it inhabits [7,8]. Earthworms that survived in the 'Seveso Disaster' (1976) ingested TCDD contaminated soils. They were shown to bio-accumulate dioxin in their tissues and concentrate it on average 14.5 fold [6].

Earthworms have also been reported to bio-accumulate 'endocrine disrupting chemicals' (EDCs) from sewage. Significantly high concentrations of EDCs (dibutylphthalate, dioctylphthalate, bisphenol-A and 17  $\beta$ -estradiol) in tissues of earthworms (*E. fetida*) living in sewage percolating filter beds and also in garden soil [9].

*E. fetida* was used as the test organisms for different soil contaminants and several reports indicated that *E. fetida* tolerated 1.5% crude oil (containing several toxic organic pollutants) and survived in this environment [10,11]. Studies shows that earthworms can tolerate and bio-accumulate high concentrations of heavy metals like cadmium (Cd), mercury (Hg), lead (Pb) copper (Cu), manganese (Mn), calcium (Ca), iron (Fe) and zinc (Zn) in their tissues without affecting their physiology and this particularly when the metals are mostly non-bioavailable. The species *Lumbricus terrestris*, *L. rubellus* and *D. rubida* was found to bio-accumulate very high levels of lead (Pb) and Cadmium (Cd) in their tissues [7].

#### 3.3. Destroy Pathogens and Disinfect the Environment

Earthworms routinely devour on the protozoa, bacteria and fungus as food in any waste materials or soil where they inhabit. They seem to realize instinctively that anaerobic bacteria and fungi are undesirable and so feed upon them preferentially, thus arresting their proliferation. More recently, Dr. Elaine Ingham has found in her research that worms living in pathogen-rich materials (e.g. sewage and sludge), when dissected, show no evidence of pathogens beyond 5 mm of their gut. This confirms that something inside the worms destroys the pathogens, and excreta (vermicast) becomes pathogen-free [5,6]. In the intestine of earthworms some bacteria & fungus (*Penicillium* and *Aspergillus*) have also been found [12]. The earthworms also release coelomic fluids that have anti-bacterial properties and destroy all pathogens in the waste biomass [13]. They produce 'antibiotics' and kills the pathogenic organisms in the waste and soil where they inhabit and render it virtually sterile. It was reported that the removal of pathogens, faecal coliforms (*E. coli*), *Salmonella* spp., enteric viruses and helminth ova from sewage and sludge appear to be much more rapid when they are processed by *E. fetida*. Of all *E. coli* and *Salmonella* are greatly reduced [14].

In another study the pathogen die-off in vermicomposting of sewage sludge spiked with *E. coli*, *S. typhimurium* and *E. faecalis* at the  $1.6-5.4 \times 10^6$  CFU/g,  $7.25 \times 10^5$  CFU/g and  $3-4 \times 10^4$  CFU/g respectively. The composting was done with different bulking materials such as lawn clippings, sawdust, sand and sludge alone for a total period of 9 months to test the pathogen safety of the product for handling. It was observed that a safe product

was achieved in 4-5 months of vermicomposting and the product remained the same quality without much reappearance of pathogens after in the remaining months of the test [15]. Other studies also confirmed significant human pathogen reduction in biosolids vermicomposted by earthworms. Pathogens like enteric viruses, parasitic eggs and *E. coli* were reduced to safe levels in sludge vermicast [16-18]. Studies also revealed that the earthworms reduced the population of *Salmonella* spp. to less than 3 CFU/gm of vermicomposted sludge. There were no fecal coliforms and *Shigella* spp. and no eggs of helminths in the treated sludge. [16,19,20].

### 3.4. Low Greenhouse Gas (GHG) Emissions by Vermicomposting of Waste with Earthworms

Emission of greenhouse gases carbon dioxide ( $\text{CO}_2$ ), methane ( $\text{CH}_4$ ) and nitrous oxide ( $\text{N}_2\text{O}$ ) in waste management programs of both garbage & sewage has become a major global issue today in the wake of increasing visible impacts of global warming. Biodegradation of organic waste either by composting or when disposed in landfills has long been known to generate methane ( $\text{CH}_4$ ) and nitrous oxide ( $\text{N}_2\text{O}$ ) resulting from the slow anaerobic decomposition of waste organics over several years [21]. Molecule to molecule,  $\text{CH}_4$  is 20-22 times and  $\text{N}_2\text{O}$  is 296-310 times more powerful GHG than the  $\text{CO}_2$ . Studies have also indicated high emissions of nitrous oxide ( $\text{N}_2\text{O}$ ) in proportion to the amount of food waste used, and methane ( $\text{CH}_4$ ) is also emitted in high amounts if the composting piles contain cattle manure. [22-25].

Studies have established that vermicomposting of wastes by earthworms significantly reduce the total emissions of greenhouse gases in terms of  $\text{CO}_2$  equivalent, especially the highly powerful GHG nitrous oxide ( $\text{N}_2\text{O}$ ). Worms significantly increase the proportion of 'aerobic to anaerobic decomposition' in the compost pile by burrowing and aerating actions leaving very few anaerobic areas in the pile, and thus resulting in a significant decrease in methane ( $\text{CH}_4$ ), nitrous oxide ( $\text{N}_2\text{O}$ ) and also volatile sulfur compounds which are readily emitted from the conventional (microbial) composting process [26]. Analysis of vermicompost samples has shown generally higher levels of available nitrogen (N) as compared to the conventional compost samples made from similar feedstock. This implies that the vermicomposting process by worms is more efficient at retaining nitrogen (N) rather than releasing it as  $\text{N}_2\text{O}$ .

Our studies also showed that on average, aerobic, anaerobic and vermicomposting systems emitted 504, 694 and 463  $\text{CO}_2\text{-e/m}^2/\text{hour}$  respectively. This is significantly much less than the landfills emission which is 3640

$\text{CO}_2\text{-e/m}^2/\text{hour}$  due to the extremely anaerobic conditions existing in the landfills. Vermicomposting emitted minimum of  $\text{N}_2\text{O}$ -1.17  $\text{mg/m}^2/\text{hour}$ , as compared to Aerobic Composting (1.48  $\text{mg/m}^2/\text{hour}$ ) and Anaerobic Composting (1.59  $\text{mg/m}^2/\text{hour}$ ). Hence, earthworms can play a good part in the strategy of greenhouse gas reduction and mitigation in the disposal of global MSW [21,27].

### 3.5. Earthworms Combat Soil Salinity & Improve Fertility of Sodic Soils

Studies indicate that *Esinea fetida* can tolerate soils nearly half as salty as seawater i.e. 15 gm/kg of soil and also improve its biology and chemistry. (Average seawater salinity is around 35 g/L). Farmers at Phaltan in Satara district of Maharashtra, India, applied live earthworms to their sugarcane crop grown on saline soils irrigated by saline ground water. The yield was 125 tones/hectare of sugarcane and there was marked improvement in soil chemistry. Within a year there was 37% more nitrogen, 66% more phosphates and 10% more potash. The chloride content was less by 46% [28,29]. In another study there was good production of potato (*Solanum tuberosum*) by application of vermicompost in a reclaimed sodic soil in India. The sodicity (ESP) of the soil was also reduced from initial 96.74 to 73.68 in just about 12 weeks. The average available nitrogen (N) content of the soil increased from initial 336.00 kg/ha to 829.33 kg/ha [30].

## 4. Role of Earthworms in Environmental Protection & Food Production

### 4.1. Safe Management of Municipal & Industrial Solid Wastes While Diverting them from Landfills & Converting into Valuable Resource (Nutritive Fertilizer)

We are facing the escalating economic and environmental cost of dealing with current and future generation of mounting municipal solid wastes. Millions of tons of MSW generated from the modern society are ending up in the landfills everyday, creating extraordinary economic and environmental problems for the local government to manage and monitor them for environmental safety (emission of greenhouse and toxic gases and leachate discharge threatening ground water contamination) [31]. Construction of secured engineered landfills incurs 20-25 million U.S. dollars before the first load of waste is dumped. According to Australian Bureau of Statistics over the past 5 years the cost of landfill disposal of waste has increased from AU \$ 29 to AU \$ 65 per ton of waste in Australia. During 2002-2003, waste management services within Australia cost AU \$ 2458.2 millions. In 2002-03 Australians generated 32.3 million tonnes of

MSW of which 17.4 mt *i.e.* about 54% ended up in landfills [32].

A serious cause of concern today is the emission of powerful greenhouse gases (GHG) resulting from the disposal of MSW either in the landfills or from their management by composting [21]. The Australian Greenhouse Office reported that disposal of MSW (primarily in landfills) contributed 17 million tonnes CO<sub>2</sub>-e of GHG in Australia in 2005, equivalent to the emissions from 4 millions cars or 2.6% of the national GHG emissions [33]. Vermicomposting of all biodegradable 'organic wastes' is emerging as a new tool in all developed world to divert large portion of community wastes (over 70%) from landfills and also reduce the GHG emissions significantly as discussed above [27].

Earthworms have real potential to both increase the rate of aerobic decomposition and composting of organic matter, and also to stabilize the organic residues in them. Earthworm participation enhances natural biodegradation and decomposition of organic waste from 60 to 80% over the conventional composting. Given the optimum conditions of temperature (20-30°C) and moisture (60-70%), about 5 kg of worms (numbering approx. 10,000) can vermicompost 1 ton of waste into vermi-compost in just 30 days [4].

#### **4.1.1. Detoxified and Disinfected Nutritive End-Products (Vermicompost)**

The earthworms ingest and bio-accumulate toxic materials, selectively devour on harmful microbes (pathogens) in the waste biomass. The end product is more homogenous, 'detoxified' and 'disinfected', richer in 'plant-available nutrients & humus' and significantly low contaminants.

#### **4.1.2. Role of Earthworms in Safe Management of Environmentally Hazardous Wastes**

Earthworms can degrade 'fly-ash' from the coal power plants which is considered as a 'hazardous waste' and poses serious disposal problem due to heavy metal contents. Earthworms ingest the heavy metals from the fly-ash while converting them into vermi-compost. It can even degrade 'human excreta' into odourless porous product with good texture and safe pathogen quality [15,34].

Worms can also vermicompost 'sewage sludge'—a great environmental hazard containing toxic chemicals and pathogens. In 12 weeks the black and brittle sludge became a homogenous and porous mass of brown vermicast with light texture. Foul odor disappeared by week 2. Upon chemical analysis, the vermicomposted sludge was over 80% free of heavy metals cadmium (Cd) and lead (Pb) and almost completely free of any pathogens [20].

### **4.2. Safe Management of Municipal and Industrial Wastewater without Formation of Sludge, their Purification, Detoxification & Disinfection for Reuse**

Vermifiltration of wastewater using waste eater earthworms is a newly conceived novel technology with several advantages over the conventional systems. Earthworms body work as a 'biofilter' and they have been found to remove the 5 days biological oxygen demand (BOD<sub>5</sub>) by over 90%, chemical oxygen demand (COD) by 80-90%, total dissolved solids (TDS) by 90-92% and the total suspended solids (TSS) by 90-95% from wastewater by the general mechanism of 'ingestion' and biodegradation of organic wastes and also by their 'absorption' through body walls. Worms also remove chemicals including heavy metals and pathogens from treated wastewater. They have the capacity to bio-accumulate high concentrations of toxic chemicals in their tissues and kill any pathogen by discharge of anti-pathogenic 'coelomic fluid' and the resulting treated wastewater becomes almost free of chemicals and pathogens to be reused for non-potable purposes [13].

### **4.3. Cleaning Up of Chemically Contaminated Lands & Soils and Making them Productive: Converting the 'Wastelands into Wonderlands'**

Large tract of arable land is being chemically contaminated due to mining activities, heavy use of agro-chemicals in farmlands, landfill disposal of toxic wastes and other developmental activities like oil and gas drilling. No farmland of world especially in the developing nations are free of toxic pesticides, mainly aldrin, chlordane, dieldrin, endrin, heptachlor, mirex and toxaphene. According to National Environment Protection Council there are over 80,000 contaminated sites in Australia. There are 40,000 contaminated sites in US; 55,000 in just six European countries and 7,800 in New Zealand. There are about 3 million contaminated sites in the Asia-Pacific. These also include the abandoned mine sites along with the closed landfills. The contaminated sites mostly contain heavy metals cadmium (Cd), lead (Pb), mercury (Hg), zinc (Zn) etc. and chlorinated compounds like the PCBs and DDT. Cleaning them up mechanically by excavating the huge mass of contaminated soils and disposing them in secured landfills will require billions of dollars. There is also great risk of their leaching underground (aggravated by heavy rains) and contaminating the groundwater. Contaminated soils and waters pose major environmental, agricultural and human health problems worldwide.

Earthworms have been found to bio-accumulate heavy

metals, pesticides and lipophilic organic micropollutants like the polycyclic aromatic hydrocarbons (PAH) from the soil. *E. fetida* was used as the test organisms for different soil contaminants and several reports indicated that *E. fetida* tolerated 1.5% crude oil (containing several toxic organic pollutants) and survived in this environment. [8,11,35,36].

Significantly, vermiremediation leads to total improvement in the quality of soil and land where the worms inhabit and make them highly productive. Earthworms significantly contribute as soil conditioner to improve the physical, chemical as well as the biological properties of the soil and its nutritive value. They swallow large amount of soil everyday, grind them in their gizzard and digest them in their intestine with aid of enzymes. Only 5-10 percent of the digested and ingested material is absorbed into the body and the rest is excreted out in soil in the form of fine mucus coated granular aggregates called 'vermicastings' which are rich in NKP (nitrates, phosphates and potash), micronutrients and beneficial soil microbes including the 'nitrogen fixers' and 'mycorrhizal fungus'.

Of considerable economic and environmental significance is that the worm feed used in vermiremediation process is necessarily an 'organic waste' product. This means that it would also lead to reuse and recycling of vast amount of organic wastes which otherwise end up in landfills for disposal at high cost. And what is of still greater economic and environmental significance is that the polluted land is not only 'cleaned-up' but also 'improved in quality'. The soil becomes lighter and porous rich in biological activities and the productivity is increased to several times. During the vermi-remediation process of soil, the population of earthworms increases significantly benefiting the soil in several ways. A 'wasteland' is transformed into 'wonderland'. Earthworms are in fact regarded as 'biological indicator' of good fertile soil and land.

#### 4.3.1. Mechanism of Worm Action in Remediation of Contaminated Soils

Earthworms uptake chemicals from the soil through passive 'absorption' of the dissolved fraction through the moist 'body wall' in the interstitial water and also by mouth and 'intestinal uptake' while the soil passes through the gut. Earthworms apparently possess a number of mechanisms for uptake, immobilization and excretion of heavy metals and other chemicals. They either 'bio-transform' or 'biodegrade' the chemical contaminants rendering them harmless in their bodies. Some metals are bound by a protein called 'metallothioneins' found in earthworms which has very high capacity to bind metals. The chloragogen cells in earthworms appear to mainly accu-

mulate heavy metals absorbed by the gut and their immobilization in the small spheroidal chloragosomes and debris vesicles that the cells contain. Earthworms have also been found to biodegrade 'toxic organic contaminants' like phthalate, phenanthrene and fluoranthene in soil [7,37].

#### 4.3.2. Role of Earthworms in Removing Chemical Contaminants from Soils

##### 1) Removal of Heavy Metals

Earthworms can bio-accumulate high concentrations of heavy metals. They can particularly ingest and accumulate extremely high amounts of zinc (Zn), lead (Pb) and cadmium (Cd). Cadmium levels up to 100 mg per kg dry weight have been found in tissues. Earthworm species *Lumbricus terrestris* can bio-accumulate in their tissues 90-180 mg lead (Pb)/gm of dry weight, while *L. rubellus* and *D. rubida* it was 2600 mg /gm and 7600 mg /gm of dry weight respectively. Zinc (Zn), manganese (Mn), and iron (Fe) were shown to be excreted through the calciferous glands of earthworms [7,38].

##### 2) Removal of Polycyclic Aromatic Hydrocarbons (PAH's)

PAHs are priority pollutants and cause great concern with respect to human health and environment. They are inherently 'recalcitrant hydrocarbons', and the higher molecular weight PAHs are very difficult to remediate. Earthworm species *L. rubellus* degraded spiked PAHs phanthrene & fluoranthene (100 µg/kg of soil). Losses of both PAHs occurred at a faster rate in soils with earthworms, than the soil without worms. After 56 days 86% of the phenanthrene was removed. *E. fetida* was also found to degrade the PAHs. The concentration of anthracene decreased by 2-fold after addition of earthworms, benzo(a)pyrene decreased by 1.4-fold and phenanthrene was completely removed (100%) by earthworms [8,37].

We studied the remedial action of earthworms on PAHs contaminated soils obtained from a former gas works site in Brisbane, Australia where gas was being produced from coal. The initial concentration of total PAHs compounds in the soil at site was greater than 11,820 mg/kg of soil. The legislative requirements for PAHs concentration in soil in Australia is only 100 mg/kg for industrial sites and 20 mg/kg for residential sites. Worms removed nearly 80% of the PAHs as compared to just 47% where worms were not applied and only microbial degradation occurred. This was just in 12 weeks study period. It could have removed by 100% in another few weeks. More significant was that the worm added soil became odor-free of chemicals in few days and were more soft and porous in texture [39].

##### 3) Removal of Petroleum and Crude Oil Hydrocarbons

Studies with earthworm species *Eisenia fetida* on oil contaminated soil revealed that worms significantly decreased oil contents in comparison to the control. It also successfully treated high molecular weight hydrocarbons 'asphaltenes' from the Prestige Oil Spill. Earthworms mineralized the asphaltenes thus eliminating it from the system. It also decontaminated complex hydrocarbons polluted soil [40-42].

#### 4) Removal of Agrochemicals

There is no farmland in world which was not contaminated with agrochemicals in the wake of 'green revolution' of 1960s which unleashed heavy use of agrochemicals to boost farm production. Several studies have found definite relationship between 'organochlorine pesticide' residues in the soil and their amount in earthworms, with an average concentration factor (in earthworm tissues) of about 9 for all compounds and doses tested. Studies indicated that the earthworms bio-accumulate or biodegrade 'organochlorine pesticide' and 'polycyclic aromatic hydrocarbons' (PAHs) residues in the medium in which it lives [7,43,44].

#### 5) Removal of Polychlorinated Biphenyls (PCBs)

PCBs are a group of oily, colorless, organic fluids belonging to the same chemical family as the pesticide DDT. They constitute a family of chemicals with over 200 types, and are used in transformers and power capacitors, electrical insulators, as hydraulic fluids and diffusion pump oil, in heat transfer applications, as plasticizers for many products. PCBs are categorized as 'unusually toxic' and 'persistent organic pollutant' (POPs). They have serious adverse effects on the human health and the environment. PCB contaminated soil treated with earthworms resulted in significantly greater PCB losses (average 52%) when compared to the soil without earthworm [45].

#### 4.3.3. Use of Earthworms in Soil Decontamination: Acquiring Global Agenda

Traditionally, remediation of chemically contaminated soils involves 'off-site' management by excavating and subsequent disposal by burial in secured landfills. This method of remediation is very costly affair and merely shifts the contamination problem elsewhere. Additionally, this involves great risk of environmental hazard while the contaminated soils are being transported and 'migration of contaminants' from landfills into adjacent lands and water bodies by leaching. Soil washing for removing inorganic contaminants from soil is another alternative to landfill burial, but this technique produce a 'residue' with very high metal contents which requires further treatment or burial.

The greatest advantage of vermiremediation technology is that it is 'on-site' treatment and there is no additional problems of 'earth-cutting', 'excavation' and 'transportation' of contaminated soils to the landfills or to the

treatment sites incurring additional economic and environmental cost. Vermiremediation would cost about \$ 500-1000 per hectare of land as compared to \$ 10,000-15,000 per hectare by mechanical excavation of contaminated soil & its landfill disposal.

Vermiremediation by commercial vermiculture in U.K. 'Land Reclamation and Improvements Programs' has become an established technology for long-term soil decontamination, improvement & maintenance, without earth-cutting, soil excavation and use of chemicals'. U.S., Australia and other developed nations are also following.

#### 4.4. Restoration of Soil Fertility to Produce Safe, Chemical-Free Food for Society without Recourse to Environmentally Destructive Agrochemicals

Earthworms lead to total improvement in the physical (soil porosity & softness), chemical (good pH and essential plant nutrients) and biological (beneficial soil microbes & organisms) quality of the soil and land where they inhabit. They 'regenerate' even the compacted soil due to burrowing actions and make it productive. Such soils allow good aeration and water percolation [46]. They swallow large amount of soil with organics (microbes, plant & animal debris) and excrete them out as 'vermicasts' which are rich in NKP (nitrates, phosphates and potash), micronutrients and beneficial soil microbes. Even after single application of vermicompost the net overall efficiency of nitrogen (N) is considerably greater than that of chemical fertilizers.

##### 4.4.1. Potential of Vermicompost to Replace the Environmentally Destructive Agro-Chemicals and Produce Chemical-Free Organic Foods

There have been several reports that earthworms and its vermicompost can induce excellent plant growth and promote good crop production without chemical fertilizers. Glasshouse studies made at CSIRO Australia found that the earthworms (*Aporrectodea trapezoids*) increased growth of wheat crops (*Triticum aestivum*) by 39%, grain yield by 35%, lifted protein value of the grain by 12% & also resisted crop diseases as compared to the control [47]. Studies on the agronomic impact of vermicompost on cherries found that it increased yield of 'cherries' for three (3) years after 'single application' inferring that the use of vermicompost in soil builds up fertility and restore its vitality for long time contrary to chemical fertilizers [48].

##### 4.4.2. Earthworms Protects Plants against Pests and Diseases & Significantly Reduce Use of Environmentally Destructive Chemical Pesticides

Earthworms are both 'plant growth promoter and protec-

tor'. There has been considerable evidence in recent years regarding the ability of earthworms and its vermicompost to protect plants against various pests and diseases either by suppressing or repelling them or by inducing biological resistance in plants to fight them or by killing them through in-built pesticidal action [49-51]. Spray of chemical pesticides is significantly reduced by over 75% where earthworms and vermicompost are used in agriculture [29].

## 5. Role of Earthworms in Protection of Human Health

Traditional medicinemen in China and Philippines used earthworms in folkloric healings of many sickness such as to cure fever, inflammation of different parts of the body, stomach-aches and toothaches, rheumatism and arthritis, to cure mumps and measles and even to make child delivery easier by faster contraction of the uterus and reducing labour pains. China has been using earthworms in traditional healing for 2,300 years [52]. The Chinese Materia Medica by Li Shizhen (1518-1593) listed 40 usage of earthworms in traditional medicine such as 'hemiplegia' (a condition where half of the body is paralysed) 'dilating blood vessels', 'lowering blood pressure', 'smoothing asthma', 'alleviating pains', 'relieving impotence', 'promoting lactation', 'protecting the skin', as anti-bacterial' & 'anti-convulsions' and as a 'tonic' [53].

### 5.1. Use of Earthworms in Development of Modern Medicine

In the last 10 years, a number of earthworm's 'clot-dissolving', 'lytic' and 'immune boosting' compounds have been isolated and tested clinically. Current researches made in Canada, China, Japan and other countries on the identification, isolation and synthesis of some 'bioactive compounds' from earthworms (*Lumbricus rubellus* & *Eisenia fetida*) with potential medicinal values have brought revolution in the vermiculture studies. Some of these compounds have been found to be enzymes exhibiting 'anti-blood clotting' effects [54].

### 5.2. Cure for Heart Diseases and Cancer

Lumbrokinase (LK) is a group of 6 'proteolytic enzymes' and recent researches suggest that it may be effective in treatment and prevention of 'ischemic heart disease' as well as 'myocardial infarction', 'thrombosis' of central vein of retina, 'embolism' of peripheral veins, and 'pulmonary embolism'. It is now being used in the treatment of 'cerebral infarction'. Japanese scientists also confirmed the curative effects of 'lumbrokinase' experimentally in the 1980s. [55]. Researches done at Ohio State University, USA, show that cancer cannot be induced in

earthworms inferring that there are some bioactive compounds and 'genetic defense' mechanism that protects them. The group of enzymes lumbrokinase (LK) also promises to wage a 'war on cancer' [56].

### 5.3. Earthworms for Production of Antibiotics

The coelomic fluid of earthworms have been reported to have anti-pathogenic activities and are good biological compound for the production of 'antibiotics' [13].

### 5.4. Combating Stress & Increasing Human Longevity

Scientists in the University of Colorado, U.S. believe that researches into earthworms may provide an insight into increasing the longevity of humans up to around 120 years. By exposing the earthworms to stress they identified the genes (biomarker of ageing) which may allow to modify humans 'stress response system' in order to extend their life.

## 6. Role of Earthworms in Production of Materials for Consumer Industries

### 6.1. Raw Materials for Rubber, Lubricant, Detergent, Soaps and Cosmetics

Some biological compounds from earthworms are also finding industrial applications. Being 'biodegradable' they are environmentally friendly and sustainable. 'Stearic acid' found in earthworms is a long chain saturated fatty acid and are widely used as 'lubricant' and as an 'additive' in industrial preparations. It is used in the manufacture of metallic stearates, pharmaceuticals soaps, cosmetics and food packaging. It is also used as a 'softner', 'accelerator activator' and 'dispersing agents' in rubbers. Industrial applications of lauric acid and its derivatives are as 'alkyd resins', 'wetting agents', a 'rubber accelerator' and 'softner' and in the manufacture of 'detergents' and 'insecticides'. Worms are also finding new uses as a source of 'collagen' for pharmaceutical industries [57,58].

### 6.2. Nutritive Feed Materials for Poultry, Dairy and Fishery Industries

Earthworms are rich in high quality protein (65%) and is 'complete protein' with all essential amino acids. There is 70-80% high quality 'lysine' and 'methionine'. Glumatic acid, leucine, lysine & arginine are higher than in fish meals. Tryptophan is 4 times higher than in blood powder and 7 times higher than in cow liver. Worms are also rich in Vitamins A & B. There is 0.25 mg of Vitamin B<sub>1</sub> and 2.3 mg of Vitamin B<sub>2</sub> in each 100 gm of earthworms. Vitamin D accounts for 0.04-0.073% of earthworms wet weight. Thus worms are wonderful pro-

biotic feed for fish, cattle and poultry industry. They are being used as 'additives' to produce 'pellet feeds' in the USA, Canada and Japan [59,60].

As earthworm protein is complete with 8-9 essential amino acids especially with the tasty 'glutamic acid' it can be used for human beings as well. Worm protein is higher than in any meat products with about 2% lower fats than in meats and ideal for human consumption.

## 7. Conclusions & Remarks

Value of earthworms in sustainable development (converting waste into resource, improving soil fertility & boosting crop productivity by vermicompost and production of some valuable life saving medicines for mankind) and environmental protection (detoxification and disinfection of wastewater, decontamination of soils and land remediation and replacing the environmentally destructive agro-chemicals in food production) has grown considerably in recent years all over the world. It is like getting 'gold from garbage' (highly nutritive biofertilizer) by vermi-composting technology; 'silver from sewage' (disinfected & detoxified water for reuse in agriculture & industries) by vermi-filtration technology; 'converting a wasteland (chemically contaminated lands) into wonderland' (fertile land) by vermi-remediation technology; harvesting 'green gold' (food crops) by using 'black gold' (vermicompost) by agro-production technology; creating a 'worm factory' to produce medicines & materials for societal use. In India, the earthworms have enhanced the lives of poor and the unemployed. Educated unemployed have now taken to vermicomposting business on commercial scale. The three versatile species *E. fetida*, *E. euginae* and *P. excavatus* performing wide social, economic & environmental functions occur almost everywhere.

And if vermicompost can 'replace' the 'chemical fertilizers' for production of 'safe organic foods' which has now been proved worldwide, it will be a giant step towards achieving global 'social, economic & environmental sustainability'. Production of chemical fertilizers is 'environmentally damaging' (generating hazardous wastes & pollutants and greenhouse gases) in its entire life-cycle, since harnessing of raw materials from the earth crust, to their processing in factories and application in farms (polluting soil & killing beneficial organisms) with severe economic & environmental implications. Production and use of 1 kg of chemical nitrogen fertilizer emits 2,500 gm of CO<sub>2</sub>, 10 gm N<sub>2</sub>O & 1 gm CH<sub>4</sub>. Molecule to molecule, N<sub>2</sub>O and CH<sub>4</sub> are 310 & 22 times more powerful GHG than CO<sub>2</sub>.

Earthworms are truly justifying the beliefs and fulfilling the dreams of Sir Charles Darwin who called earthworms as '*unheralded soldiers of mankind*' and '*friends*

*of farmers*'. Darwin wrote that '*no other creature on earth has done so much for mankind*' as the earthworms.

It is also justifying the beliefs of Dr. Anatoly Igonin one of the great contemporary vermiculture scientist from Russia who said '*Earthworms create soil & improve soil's fertility and provides critical biosphere's functions: disinfecting, neutralizing, protective and productive*'.

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# Bioefficacy and Residue Studies of Fantac (Biostimulant) in Rice Crop under Sub-Tropical Conditions

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## ABSTRACT

Field investigations were conducted for three seasons from July 2006-2007 to test the efficacy of Fantac (N-ATCA) in transplanted rice and also determine its harvest time residues in paddy grain, straw, husk and soil by HPLC. It was applied at the rate of 25, 50, 75 and 100 mL/100 L respectively. The results revealed that foliar spray of this agrochemical on rice crop did not produce any significant effect on the general growth parameters but increased the number of grains/panicle. The residues of fantac were found to be < 0.05 µg/g at the time of harvest which are below MRL and thus Fantac can be considered as environmentally safe.

**Keywords:** Fantac, Biostimulant, Harvest, Residues, HPLC

## 1. Introduction

Biostimulants are referred to as positive plant growth regulators or metabolic enhancers which when applied in small quantities enhance the growth and overall development of plants. One such biostimulant is Fantac, a mixture of 5% N-Acetyl thiazolidine carboxylic acid (N-ATCA) and 0.1% folic acid has been recently introduced by Coromandel Fertilizers, Secunderabad, India for attaining higher values of yield attributes in rice. It is a stabilizer buffer which on application to plants helps them to sustain stresses more effectively and enhance crop yields both in quality and quantity [1-3]. It also produces specific effects on the physiological processes of plants [4].

Though biostimulants serve to be very effective in plant growth and enhancing crop yield, but since soil acts as an ultimate sink for all the chemicals applied on it, therefore even slightly higher concentrations of any chemical can harm the next crop in rotation or may also leach down to contaminate ground water resources. Recommendations for the use of agrochemicals on a crop cannot be made until its residue studies have been carried out [5]. Hence a detailed study was therefore conducted to evaluate the effect of Fantac on rice and also estimate

the harvest time residues of this biostimulant in paddy (grain, straw, husk) and soil with a view to ensure human and environmental safety.

## 2. Materials and Methods

### 2.1. Procurement of Chemicals

The technical grade Fantac (99.9% purity) was obtained from M/S Coromandel Fertilizers Pvt. Ltd., Secunderabad, India. All the other reagents and chemicals were procured from Merck, India Ltd.

### 2.2. Field Experiment

Bioefficacy field trials were conducted for three seasons during the year 2006-2007 on rice (cv. Pant Dhan-4) in randomised block design at Crop Research Centre, G.B. Pant University, Pantnagar with three replications and five treatments including untreated control. The crop was sowed in 6 m × 6 m plot in June 2006 and the transplanting was done after 30 days of sowing. Application of Fantac at the rate of 25, 50, 75 and 100 mL/100 L water was done using a knapsack sprayer at tillering stage (1<sup>st</sup> application), panicle initiation stage (2<sup>nd</sup> application) and 15 days after the second application (last application).

The observations for the plant height, tillers per plant, panicle length, grains per panicle, grain and straw yield were taken. The phytotoxicity of fantac was also determined as per CIB guidelines on 0-10 scale, by comparing the toxicity symptoms on fifty days old plants from the treated and untreated plots.

For residue studies the samples of paddy grain, straw, husk and soil treated with Fantac at the rate of 75 and 100 mL/100 L water were collected from each plot at the time of harvest and extracted for its residues.

### 2.3. Extraction and Cleanup

**Paddy samples:** The well ground rice grain, chopped straw and husk samples (50 g each) in triplicate were extracted twice with methanol and filtered. The combined filtrate was partitioned with isooctane and 0.01% t-butanol in dichloromethane mixture. The aqueous phase was extracted twice and passed through anhydrous sodium sulfate to remove any traces of water. The organic layer was concentrated in a Rotavapor and the residue was dissolved in methanol: acetonitrile mixture (1:1). The dissolved residue was cleaned up by column chromatography using activated silica gel as an adsorbent. The column was eluted with acetonitrile: methanol mixture (1:1) and again evaporated to dryness followed by reconstitution of the residue in the mobile phase.

**Soil:** Soil samples were air dried and sieved through a 2 mm sieve. Representative samples (50 g) were extracted twice with methanol and filtered. The combined filtrate was subjected to liquid partitioning with isooctane and 0.01% t-butanol in dichloromethane mixture and then cleaned up by passing through a column packed with silica gel. The column eluent was dried and dissolved in the mobile phase for HPLC analysis.

**Recovery studies:** Recovery studies were also performed by spiking all the samples with two levels of fortification of Fantac ( $0.05 \mu\text{g g}^{-1}$  and  $0.5 \mu\text{g g}^{-1}$ ) and adopting the same procedure as discussed above in order to validate the efficiency of the method of the analytical method. The samples were extracted and cleaned up following the procedure described in preceding section.

### 2.4. Residue Analysis

A Waters HPLC system with varying wavelength detector was used for chromatographic analysis. The operating parameters were column: C-18, mobile phase acetonitrile sodium dihydrogen orthophosphate buffer (6:94 v/v), pH 2.5 in isocratic mode at a flow rate of 1.0 mL/min. and detection at 210 nm.

## 3. Results and Discussion

No phytotoxic symptoms necrosis, epinasty, hyponasty,

leaf tip injury, leaf surface injury, wilting and vein clearing were observed in any of the treated plots even for 10 d after the foliar spray of this chemical. The pooled data on the effect of Fantac and other growth promoters on growth, yield attributes and grain and straw yields of rice (var. PD-4) are presented in **Table 1**. It is clearly evident from the data that foliar spray of this agrochemical on rice crop did not have any significant effect on the general growth parameters like plant height and number of tillers/plant. The yield attribute like the average panicle length was also not significantly influenced by the foliar sprays of this chemical. However, foliar spray of Fantac at the rate of 25, 50 and 75 mL/100 L water increased the number of grains/panicle by 4.8, 6.2 and 6.6 percent over control, respectively but Fantac at the rate of 50 and 100 mL/100 L water decreased the number of chaffy grains/panicle significantly by 8.1 and 13.6 percent over control, respectively. As regards the grain yield of rice, foliar spray of fantac at the rate of 25, 50, 75 and 100 mL/100 L water increased it significantly by 11.3, 17.3, 16.6 and 16.2 percent over control, respectively. Fantac alone or in combination with trace element mixture has been reported for the highest grain/seed yields in wheat [6]. In case of fruits, Fantac has been reported to enhance the photosynthetic activity and produce a significant increase in yield and size of apples [7]. Dubravec has reported an increase in the cluster number and yield of grapevine [8]. In strawberries too, an increase in the berry weight and yield has been reported [9]. The straw yield of rice in this experiment was however not significantly influenced by different treatments.

Recovery studies were done by fortifying known amount of Fantac standards and estimation of the residues left, as per the method outlined earlier. The percent recovery values of Fantac from all the samples were found to be 90.2 to 92.0 percent at higher fortification rate and 82.6 to 85.0 percent at lower rate of fortification, respectively. Standard deviation associated with the determinations ranged from 2.6 to 4.8% and the LOQ and LOD values for fantac were 0.05 and  $0.02 \mu\text{g g}^{-1}$  respectively. The study revealed that the mean recovery percentages were 87.0 for soil, 90.8 for paddy grain and 92.0 for rice straw respectively, which indicates that the method adopted for estimation of Fantac is quite suitable for extraction.

The fantac residues were also estimated in the harvested samples of paddy grain, straw and soil (**Table 2**). The fate and behavior of Fantac in soil has also been observed and since it is easily degraded by microorganisms in the soil its half life is very short [10,11]. The residue levels in the present study at harvest time were  $0.05 \mu\text{g g}^{-1}$  which is below the toxicity level in all *i.e.* paddy grain, straw and soil. Hence it can be considered safe both for

**Table 1. Effect of foliar spray of growth promoters on growth parameters and yield of rice (pooled data of three season).**

Treatments*	Plant height (cm)	Tillers/plants	Panicle length (cm)	Grain/panicle	Chaffy gain/panicle	Grain yield (q/h)	Straw yield (q/h)
T1	80.2	11.3	24.7	112	12	50.78	67.08
T2	80.4	11.5	23.7	114	11	53.54	63.09
T3	79.8	11.5	23.7	114	12	53.22	63.20
T4	79.4	12.2	23.3	111	10	53.04	66.32
T5	79.3	11.5	24.2	107	13	45.63	61.87
S.Em.	0.7	0.3	0.4	1.8	0.8	1.06	1.44
CD (p = 0.05)	NS	NS	NS	5	2	3.02	NS
C.V. (%)	3.6	8.81	4.31	4.8	19.06	6.16	6.69

\*(T1, T2, T3, T4 and T5 are treatments of Fantac @ 0, 25, 50, 75 and 100 mL/100 L of water)

**Table 2. Harvest time residues of Fantac in rice.**

Treatment	Rice grain	Rice straw	Soil
Fantac @ 75 mL/100 L	BDL	BDL	BDL
Fantac @ 100 mL/100 L	BDL	BDL	BDL

BDL: Below detection limit (< 0.05 ppm)

the present as well as the succeeding crop.

#### 4. Conclusions

It can be concluded from the present investigation that the growth promoter, Fantac helped in better seed setting which resulted in higher grain yields of rice. The simple extraction and clean up procedure developed for Fantac analysis in paddy grain, straw and soil was found to yield good recovery of the biostimulant and it is safe from environmental and health point of view as its residues do not persist both in crop and soil at the harvest time.

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# Spatial and Temporal Variation of Urban Air Quality: A GIS Approach

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## ABSTRACT

*This study investigated the seasonal variation of ambient air quality status of Burdwan town using GIS approach. Concentration of SO<sub>2</sub> (sulphur dioxide), NO<sub>2</sub> (nitrogen dioxide) and RSPM (respiratory suspended particulate matter) were measured once a week for 24 hour in both premonsoon and postmonsoon season. The seasonal average concentration of the RSPM, SO<sub>2</sub> and NO<sub>2</sub> in premonsoon season was observed to be  $188.56 \pm 88.63$ ,  $5.12 \pm 6.27$  and  $92.51 \pm 64.78$   $\mu\text{g}/\text{m}^3$  respectively whereas in postmonsoon it was  $53.03 \pm 38.27$ ,  $8.51 \pm 7.11$  and  $162.85 \pm 184.80$   $\mu\text{g}/\text{m}^3$  respectively. Statistical analysis showed the significant monsoonal effect on mean difference of RSPM, SO<sub>2</sub> and NO<sub>2</sub> concentration. Postmonsoon concentration of ambient SO<sub>2</sub> and NO<sub>2</sub> were observed to be higher than premonsoon, suggesting longer residence times of these pollutants in the atmosphere due to stagnant conditions and low mixing height. Spatial distribution of pollutants throughout the town in both the season was represented by digital elevation model (DEM). On the basis of Air Quality Index (AQI) a GIS based air pollution surface models were generated in both the seasons by means of Inverse Distance Interpolation (IDINT) technique. From the output surface model it was found that in comparison to premonsoon there was a significant increase of clean and fairly clean area and decrease of moderately polluted area of the town during postmonsoon.*

**Keywords:** Ambient Air Quality, Seasonal Variation, Air Quality Index (AQI), Geographic Information System (GIS)

## 1. Introduction

Throughout the world, air pollution is a matter of concern at all levels. The worldwide epidemiological study on the effect of air pollution had revealed that gaseous pollutants and particulate matter had enough potential to cause severe health effect like respiratory, cardiovascular diseases and cardio pulmonary mortality [1,2]. Being a serious matter of concern now-a-day, a systematic monitoring programme all over the world especially in urban cities are urgently needed as the level of air pollution is increasing rapidly in many areas of mega cities of the developing world [3]. It was found that the modernization and industrialization of developing countries had led to the increase use of fossil fuels and their derivatives. As such, developing countries were confronted with the great challenge of controlling the atmospheric pollution especially in the rapidly growing mega cities. Concern about air pollution in urban regions is receiving increasing importance world-wide, especially pollution by gase-

ous and particulate trace metals [4-7]. The urban centers might be viewed as dense sources of enormous anthropogenic emissions of pollutants, which could alter the atmospheric composition, chemistry and life cycles in its down wind regimes, extending over several hundred kilometers [8]. It had been found that world motor vehicle population growth had reached 700 million in the year 2000 [9]. Petrol and diesels engines of motor vehicles were found to emit a wide variety pollutants, principally, oxide of nitrogen (NO<sub>x</sub>) which had an increasing impact on urban air quality [10]. Various monitoring programme had already been done in developing countries like Bangladesh and Pakistan [11,12].

In India, air pollution had also become a topic of intense debate at all levels mainly because of the enhanced anthropogenic activities [13]. Today India is one of the first ten industrial countries of the world [14]. Urban air pollution in India had increased rapidly with the population growth, numbers of motor vehicles, use of fuels with poor environmental performance, badly mentioned tran-

sportation systems, poor land use pattern, and above all, ineffective environmental regulations [8,9]. Among the worst air city the name of the capital of India is enlisted followed by Beijing, China, Xian, Kathmandu, Dhaka in Asia. So far various research work had been done on spatial and temporal variation of urban air pollution in various cities of India like Kolkata, Delhi, Lucknow, Haryana, Chennai, Mumbai, Dhanbad-Jharia and on Raniganj-Asansol [8,13,15-20].

GIS is used as a platform for spatio-temporal analysis or for building relationships between the GIS database and stand-alone modeling tools. Air data are generally very complex to model due to the underlying correlation among several pollutants. The significant differences among the results obtained from the techniques, indicated that proper air quality management requires sensitive air quality evaluation [21]. Various research work had also been done on the GIS aspect of spatio-temporal analysis of urban air quality [22-25].

Burdwan being a city of West Bengal state in eastern India and headquarters of Burdwan district now a day draws attention with respect to ambient air quality status. Not only being a busy town (populated by 2,85,871 people as per 2001 census) but also being nearest to Durgapur, this place was given importance keeping in mind that Durgapur is the 7<sup>th</sup> polluted city in India and air pollutants had the capacity to travel a long distance. Apart from several residential projects a major public private project “the largest health city of Asia” was also proposed here. Medical report (**Table 1**), collected from the Govt. Hospital of this town, reflected that health problem due to air pollution is increasing day by day. So far no systematic air quality-monitoring programme with GIS approach was reported from this town. Hence the quality of ambient air deserved a systematic as well as scientific investigations so that proper strategies could be taken to mitigate in case of any pollution was found. The objective of this study was to evaluate the premonsoon and postmonsoon distribution of selected gaseous pollutants *i.e.* sulphur dioxide (SO<sub>2</sub>), nitrogen dioxide (NO<sub>2</sub>) and respiratory suspended particulate matter (RSPM) and its interaction with meteorological parameters. This study also performed to develop a GIS based air pollution surface model on the basis of air quality index (AQI) by using continuous surface generation technique.

## 2. Methodology

### 2.1. Study Area

Burdwan town is located at 23.25° N latitude and 87.85° E longitude. It has an average elevation of 40 meters (131 feet). The city is situated a little less than 100 km north-west of Kolkata on the Grand Trunk Road (NH-2) and

**Table 1. Medical record (2008) of respiratory disease in Burdwan municipality.**

Sl. No	Month	Case	Death
1.	January	17	03
2.	February	12	01
3.	March	34	00
4.	April	09	01
5.	May	22	00
6.	June	19	05
7.	July	18	01
8.	August	04	01
9.	September	09	00
10.	October	16	01
11.	November	39	03
12.	December	30	00

\*Data Obtained from Medical Record Department Burdwan Medical College & Hospital Burdwan, West Bengal.

eastern railway (**Figure 1**). It is a city with an increasing number people opting for better residential spaces and higher living standards. The number of registered motor vehicle in the town (according to 2007 statistics) was 3, 97,5509. On basis of land use/land cover classification map (**Figure 2**) the respective locations of sampling encompassing sensitive, residential and industrial areas were selected. Altogether 25 locations encompassing all the three areas were selected randomly for air quality monitoring (**Figure 2**). Details of the sampling locations are represented in **Table 2**. Mainly the schools, colleges, university and children parks are enlisted as sensitive zones whereas the places beside road and others residential areas are considered as residential zones and others as per Central Pollution Control Board (CPCB). The place where the industries (mainly rice mills in the study area) are aggregated is considered here as industrial zone.

### 2.2. Sampling (6:00 A.M. to 6:00 A.M.) and Analysis of Gaseous Pollutants and Particulate Matter

In both the seasons the sampling was done for twenty-four (24) hours at each site. The seasonal classification was followed as per specification laid by Indian meteorological department [26]. March, April and May months were considered as premonsoon season and June, July, August, and September were considered as postmonsoon season. Air quality parameter such as repairable suspended



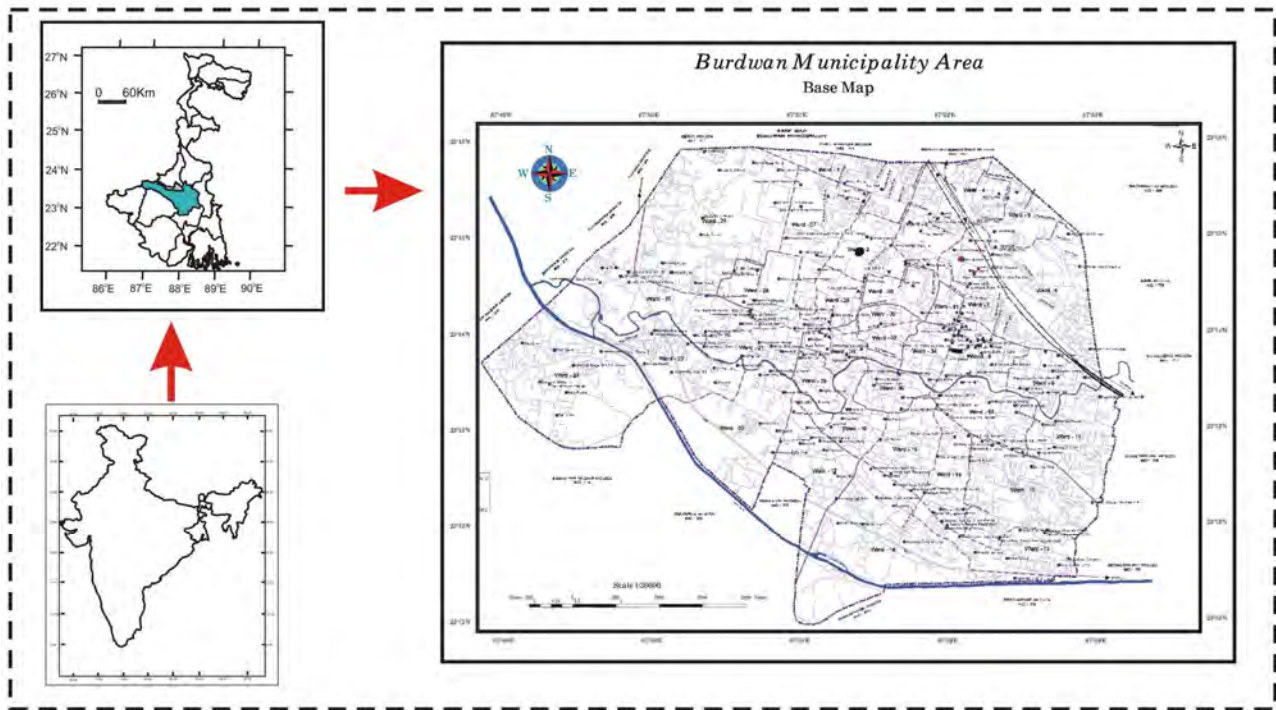


Figure 1. Study area location.

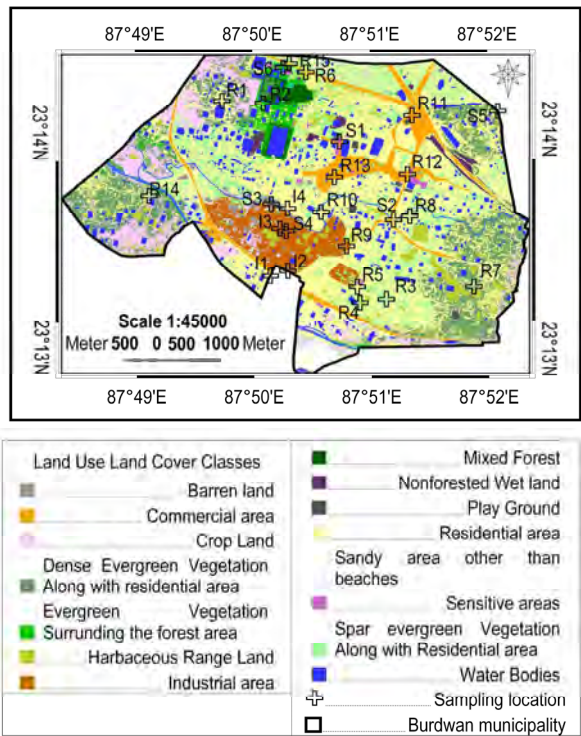


Figure 2. Land use/land cover map showing various sampling locations.

particulate matter (RSPM) which was also known as  $PM_{10}$

Table 2. Details of sampling sites.

Sampling Sites	Description
R11, R12, R13, R15, R6, R7	Residential area with high traffic density
R3, R4, R5, R8, R14	Residential area with moderate traffic density
R1, R2, R10	Residential area with low traffic density
R9	Residential area influenced by industrial emission
I1 and I2	Industrial area with high traffic density
I4	Industrial area with moderate traffic density
I3	Industrial area with low traffic density
S2, S5, S6	Sensitive area with high traffic density
S1	Sensitive area with moderate traffic density
S3	Sensitive area with moderate traffic density and highly influenced by industrial emission
S4	Sensitive area with high traffic and highly influenced by industrial emission

was monitored by using High Volume Sampler (MODEL NPM HVS) following standard procedure by IS: 5182 (Part iv). Glass fiber filter paper, popularly known as GF/A filter paper was used and the flow rate was kept at 1-1.5  $m^3/min$ . The model NPM HVS had a cyclone separator, which separated the coarser particulate matter larger than 10  $\mu m$  from air stream (drawn into the HVS)



before filtering on GF/A filter paper. Air was also allowed to pass through two impingers having specific absorbing reagent for SO<sub>2</sub> and NO<sub>2</sub>. SO<sub>2</sub> and NO<sub>2</sub> were collected by bubbling the sample in specific absorbing reagents of 25 ml put in two impingers. The average flow rate through the impingers was 0.5 l/min. After the sampling the impinger samples were kept in iceboxes and transferred to a freeze until the analysis was done. Sodium tetrachloromercurate and Sodium hydroxide were used as absorbing reagents for SO<sub>2</sub> and NO<sub>2</sub> respectively to arrest SO<sub>2</sub> in the form of dichlorosulfitomercurate complex measured spectrophotometrically at 560 nm and NO<sub>2</sub> as sodium nitrite measured at 540 nm. For analysis of SO<sub>2</sub> and NO<sub>2</sub> by spectrophotometric method, described in IS: 5182 (Part ii) and IS: 5182 (Part vi) were followed [27,28]. National ambient air quality standard (NAAQS) is represented in **Table 3**.

### 2.3. Meteorology

In each sampling location meteorological parameters such as humidity, temperature, wind speed, wind direction and rainfall were recorded both in premonsoon and postmonsoon seasons. Humidity and temperature were measured by a portable hygrometer (Model-HTC-1), rainfall is measured by a digital rain gauge (Model-RGR 126; Make-Oregon) meter whereas wind speed and direction is measured by a digital anemometer along with wind vane (Model-Lutron-AM-4201). For both the seasons two windrose diagrams (**Figures 3(a)** and **(b)**) were prepared by using windrose pro software. Apart from wind velocity and direction other meteorological data of the study area during monitoring period is represented in **Table 4**.

### 2.4. Air Quality Index (AQI)

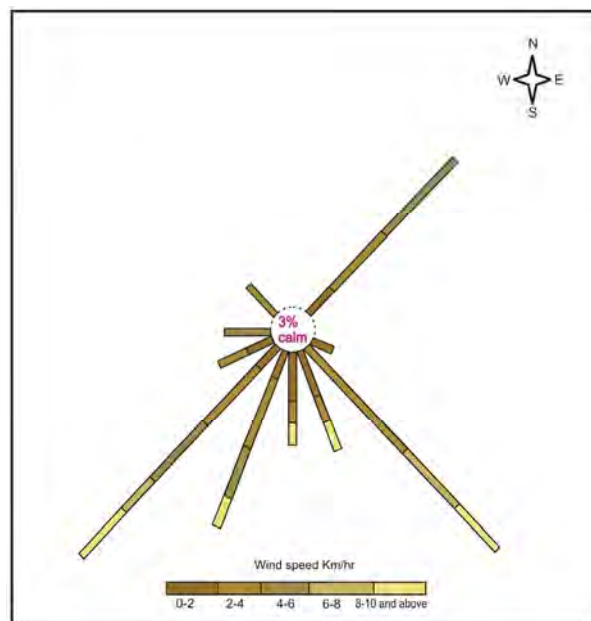
An AQI could be defined as a scheme that transforms the (weighted) values of individual air pollution related parameters into single number. Air quality index [29] was also measured here for each place in each zone. At first air quality rating of each parameter used for monitoring is calculated in each zone by the formula as;

1)  $q = 100 \times V/V_s$ ; where  $q$  = quality rating;  $V$  = observed value of parameter;  $V_s$  = value recommended for that parameter.

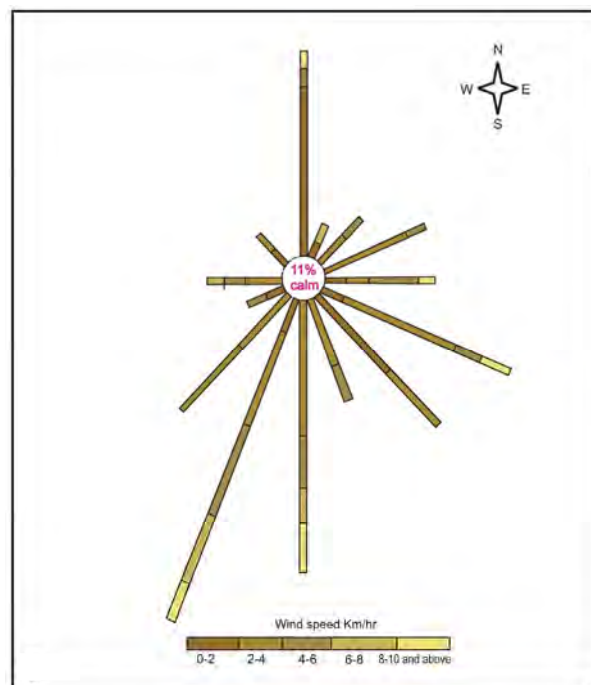
If total 'n' no of parameters were considered for air monitoring, then geometric mean of these 'n' number of quality ratings was calculated in the following way:

2)  $g = \text{anti log } \{ (\log a + \log b + \dots \dots \dots \log x)/n \}$ ; where  $g$  = geometric mean;  $a, b, c, d, x$  = different values of air quality rating; and  $n$  = number of values of air quality rating,  $\log$  = logarithm.

Air quality status [30] on the basis of AQI is represented in **Table 5**.



(a)



(b)

**Figure 3. Windrose diagram. (a) Premonsoon; (b) Postmonsoon.**

### 2.5. Statistical Analysis

#### 2.5.1. Pearson Correlation Coefficient

The Pearson correlation among SO<sub>2</sub>, NO<sub>2</sub> and RSPM was calculated by using the following formula

**Table 3. National ambient air quality standards.**

Pollutants ( $\mu\text{g}/\text{m}^3$ )	Time weighted	Concentration in ambient air in average		
		Sensitive	Industrial	Residential and others
Respirable Suspended Particulate Matter (RSPM)	24h	75	150	100
Sulphur dioxide ( $\text{SO}_2$ )	24h	30	120	80
Oxides of nitrogen ( $\text{NO}_2$ )	24h	30	120	80

Source: Central pollution control board, Delhi, 1994

**Table 4. Meteorological condition during premonsoon and postmonsoon season.**

Meteorological parameters	Premonsoon			Postmonsoon		
	Maximum	Minimum	Average	Maximum	Minimum	Average
Rainfall (mm)	118.7	42.7	78.8	433.6	228.2	324.2
Humidity (%)	75	42.5	58.22	85	53	66.13
Temperature ( $^{\circ}\text{C}$ )	35.5	16.43	24.54	33.5	16.2	23.81

**Table 5. Air quality index table (Mudri, 1999).**

Category	AQI of ambient air	Description of ambient air quality
I	Below 10	Very clean
II	Between 10-25	Clean
III	Between 25-50	Fairly clean
IV	Between 50-75	Moderately Polluted
V	Between 75-100	Polluted
VI	Between 100-125	Heavily polluted
VII	Above 125	Severely polluted

$$r = \frac{\sum_{i=1}^n (X_i - \bar{X})(Y_i - \bar{Y})}{(n-1) S_x S_y}$$

where  $X$  and  $Y$  are two variables, with means  $\bar{X}$  and  $\bar{Y}$  respectively with standard deviations  $S_x$  and  $S_y$ . Statistical significance of  $r$  value is calculated by  $t$ -test.

### 2.5.2 Student's t-Test for Difference of Means

A student's ( $t$ ) test [31] was carried out for testing significant difference between means of factors for pre- and postmonsoon periods against left sided alternative hypothesis, *i.e.*, the mean of premonsoon is less than that of the other. The test statistic, which follows  $t$ -distribution with  $(n_1 + n_2 - 2)$  degrees of freedom, is given by  $t = (X_1 - X_2) / \sqrt{(S_p^2 / n_1 - 1) + (S_p^2 / n_2 - 1)}$ ; where  $S_p^2 = (n_1 S_1^2 + n_2 S_2^2) / (n_1 + n_2)$ .

$X_1$  is the mean variable of premonsoon,  $X_2$  is the mean

variable of post-monsoon,  $S_p^2$  is the variance of combined sample (Standard Error of difference between means of pre- and postmonsoon parameters),  $n_1$  is the number of observations on variable of premonsoon and  $n_2$  is the number of observations on variable of postmonsoon. If computed value is greater than critical value there is significant difference between means.

## 2.6. RS and GIS Methodology

The following RS and GIS methodologies were adopted for carrying out the research work.

### 2.6.1. Supervised Classification of Study Area

Supervised classification of the Burdwan town was performed with the help of Resourcesat-1 satellite image and Geomatica V.10.2 software. Map collected from Burdwan municipality was considered as base map (**Figure 1**). Base map was georeferenced at latitude/longitude projection system with a datum level of India-Nepal (D076) with an output pixel spacing of 0d00'00.1900". For georeferencing ground control points (GCPs) were collected from study area by using Germin 12 GPS receiver. Burdwan municipality area was clipped from the satellite imagery and image to image georeferencing was done by using already georeferenced base map. Then supervised classification was run by using maximum likelihood classifier with null class. Thereafter both landuse/landcover and base maps were reprojected to Universal Transverse Mercator Projection (UTM) system. Twenty five (25) air sampling locations were then downloaded to the classified image from GPS through Mapsource software. Locational details along with different air quality parameters and their concentrations were at-

tached to this 25 spatial data as an attribute data.

### 2.6.2. Digital Elevation Model (DEM)

DEM is generated on the basis of sampling points, stored as a point layer along with attributes such as RSPM, SO<sub>2</sub> and NO<sub>2</sub> etc. DEM is generated by using VEDIMINT algorithm in the Geomatica V.10.1 software. The output DEM is represented as a zonation map of the said parameters (**Figures 5, 6 and 7**). The algorithm consists of three major steps plus an optional step for processing 2D features. In the first step, input vector points (RSPM, SO<sub>2</sub> and NO<sub>2</sub> concentration with respect to different locations) are reprojected to the raster coordinates and burned into the raster buffer, with the elevations generated due to different concentration of the said parameters interpolated linearly between vector nodes. 2D layers are ignored in this stage. If multiple elevation values are scanned into a single pixel, the maximum value is assigned the pixel, and the pixel is marked as a cliff. In the second step, the elevation at each DEM pixel is interpolated from the source elevation data. The interpolation process is based on an algorithm called Distance Transform. Interpolation is made between the source elevations and elevations at equal-distance points from source locations. If 2D vector layers are present, they are scan converted into a flag buffer during the optional step. The 2D features are also initialized to prepare for use in the smoothing stage. In step 3, a finite difference method is used to iteratively smooth the DEM grid. The algorithm uses over relaxation technique to accelerate the convergence. During the iterations, the source elevation values are never changed, while the interpolated values are updated based on the neighborhood values.

### 2.6.3. Inverse Distance Interpolation (IDINT)

Inverse distance interpolation is used to read the gray level values for an arbitrary number of pixel locations in order to generate a raster image based upon interpolation between the specified gray levels. This method of interpolation combines the idea of Thiessen polygon with the gradual change of trend surface. It considers weighted moving average. Weights are computed from a linear

function of distance between sets of points and the points to be predicted. In this method the size of the starting radius is specified, which defines the starting search area for interpolation points around grid point.

## 3. Results and Discussion

Results of premonsoon and postmonsoon ambient air quality status of different monitoring sites of study area encompassing industrial, residential and sensitive areas are represented in **Tables 6, 7 and 8** respectively whereas the average seasonal values of RSPM, SO<sub>2</sub> and NO<sub>2</sub> are represented in **Table 9**.

### 3.1. RSPM Scenario

During premonsoon all the industrial sites had high level of RSPM than the standard prescribed by NAAQS. This might be due to resuspension of road dust, soil dust, and vehicular traffic and nearby industrial emission [8]. But during postmonsoon RSPM level in these sites lied well below the prescribed limit. This implied that the monsoon in these sites had a major role in washing out of RSPM [20].

In residential sites RSPM level exceeded its standard in every monitoring site except R7, R13, and R15 where the level of RSPM lied very near to the standard. But in postmonsoon opposite phenomenon was observed. Only 12% of residential sites *i.e.* R11, R12, and R15 have higher level of RSPM than the permissible standard. In general it is found that most of the postmonsoon RSPM concentration was significantly less than the premonsoon concentration except site R15. This phenomenon might be corroborated to monsoonal wash out of the particles [8]. The site R15 was situated just beside National Highway. So, at that particular time of monitoring high density of traffic, road dust etc might cause it to be more negating the effect of rain which was supported by similar observation of a research work [32].

Regarding sensitive sites except site S5 and S6 most of the RSPM value lied above the limit of NAAQS standard in premonsoon while in postmonsoon the level exceeds

**Table 6. Premonsoon and postmonsoon ambient air quality status in various industrial locations of Burdwan municipality (Except AQI, all values are expressed in  $\mu\text{g}/\text{m}^3$ ).**

Sites	RSPM		SO <sub>2</sub>		NO <sub>2</sub>		AQI		Status	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
I1	173.9	74	1.59	20.34	26.62	125.78	15.05	44.33	Clean	Fairly clean
I2	326.2	53.7	26.69	23.11	66.06	60.29	64.33	32.59	Moderately polluted	Fairly clean
I3	154.6	55.2	8.6	0.38	20.19	98.6	23.16	9.80	Clean	Very clean
I4	231.1	60.41	19.46	7.51	56.09	23.91	48.88	17.12	Fairly clean	Clean

**Table 7. Premonsoon and postmonsoon ambient air quality status in various residential locations of Burdwan municipality (Except AQI, all values are expressed in  $\mu\text{g}/\text{m}^3$ ).**

Sites	RSPM		SO <sub>2</sub>		NO <sub>2</sub>		AQI		Status	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
R1	203.30	22.80	4.37	7.06	238.19	44.05	69.15	22.29	Moderately polluted	Clean
R2	174.70	15.46	0.88	1.47	161.79	90.73	33.87	14.76	Fairly clean	Clean
R3	230.00	34.26	2.13	BDL	109.74	230.51	43.79	16.60	Fairly clean	Clean
R4	303.30	38.92	0.34	10.60	29.19	163.69	16.75	47.25	Clean	Fairly clean
R5	267.30	71.80	2.21	6.95	63.15	191.21	38.77	71.37	Fairly clean	Moderately Polluted
R6	285.80	16.30	3.18	12.53	87.16	168.70	49.83	37.76	Fairly clean	Fairly clean
R7	99.40	40.97	0.6	9.11	36.77	129.66	15.04	42.29	Clean	Fairly clean
R8	135.17	32.63	0.34	3.94	88.36	193.90	18.51	33.90	Clean	Fairly clean
R9	119.16	10.00	3.17	20.34	63.04	207.12	33.38	40.38	Fairly clean	Fairly clean
R10	137.77	69.62	4.37	16.95	220.87	426.28	59.22	92.29	Moderately polluted	Polluted
R 11	323.10	112.80	6.93	5.45	140.43	79.05	78.90	42.35	Polluted	Fairly clean
R12	264.40	102.46	3.22	5.60	3.79	166.70	17.15	53.07	Clean	Moderately polluted
R13	69.61	47.16	2.69	1.32	16.10	30.34	16.76	14.34	Clean	Clean
R14	168.90	7.11	9.75	4.55	54.71	45.97	52.02	16.62	Moderately polluted	Clean
R15	96.54	141.00	2.72	6.00	158.82	97.99	40.24	50.60	Fairly clean	Moderately polluted

**Table 8. Premonsoon and postmonsoon ambient air quality status in various sensitive locations of Burdwan municipality (Except AQI, all values are expressed in  $\mu\text{g}/\text{m}^3$ ).**

Sites	RSPM		SO <sub>2</sub>		NO <sub>2</sub>		AQI		Status	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
S1	123.60	10.95	2.68	BDL	77.49	39.94	72.45	13.49	Moderately polluted	Clean
S2	191.70	45.60	2.64	7.30	158.58	207.11	105.94	100.79	Heavily polluted	Heavily polluted
S3	156.40	130.45	12.71	12.94	62.62	48.98	122.63	106.99	Heavily polluted	Heavily polluted
S4	363.60	87.15	2.72	21.96	101.46	209.28	114.13	181.04	Heavily polluted	Severely polluted
S5	60.00	40.62	0.57	3.80	79.57	937.25	34.29	128.93	Fairly clean	Severely polluted
S6	54.45	4.48	3.50	3.42	191.87	54.33	81.52	23.10	Polluted	Clean

only at site S3 and S4. This might be due to their locational disadvantages as because both these two places were located in the region where majority rice mills factories of the town are situated. So, in spite of monsoonal wash out of dust particle they reflected a high level of RSPM than the standard. Maximum RSPM level during premonsoon was found in S4 site, which was not only

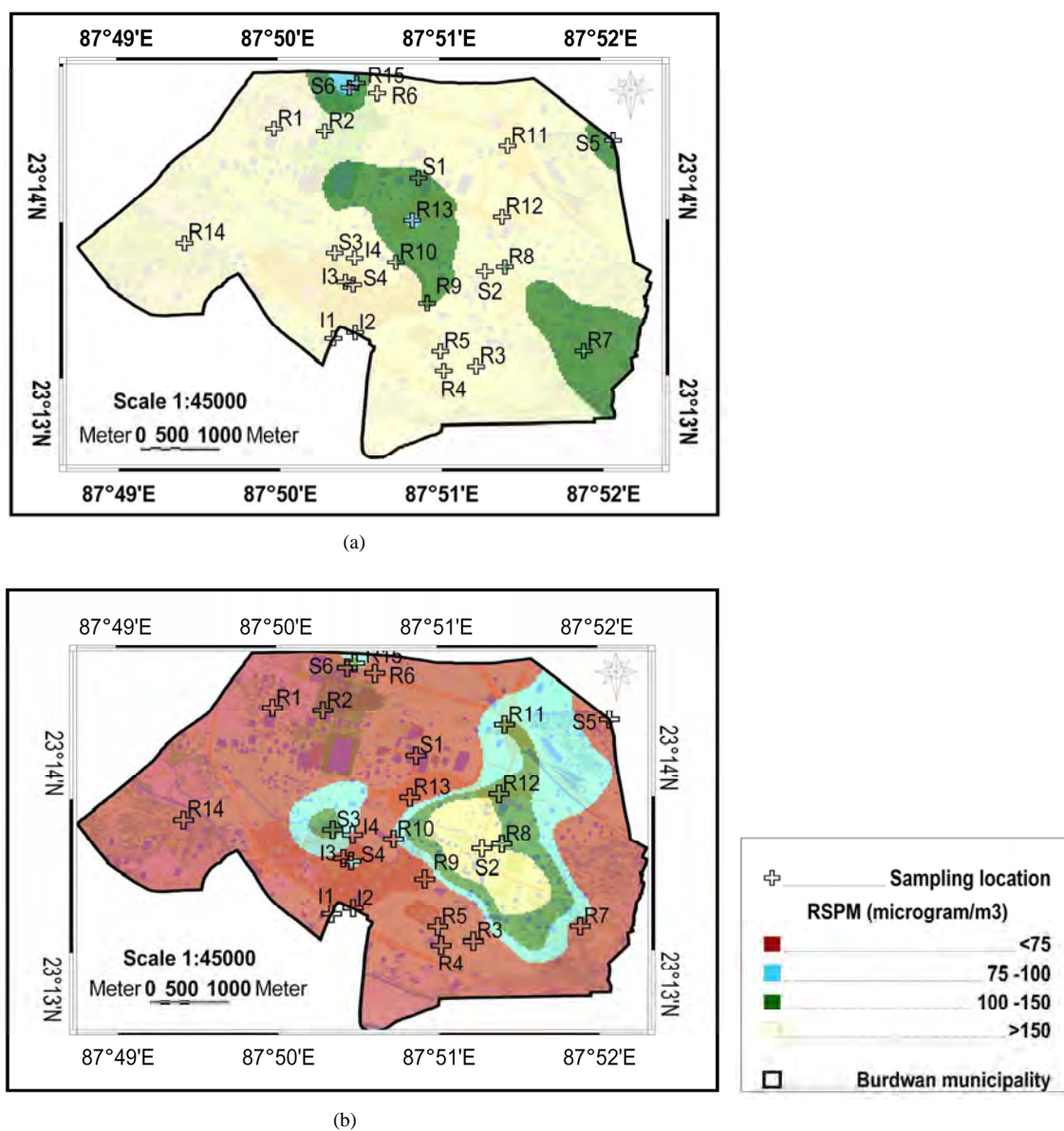
beside rice mills but also beside a main road. So, such a high level of RSPM might be attributed to resuspension of road dust, soil dust and vehicular traffic and nearby industrial emission.

Digital elevation model (DEM) with respect spatio-temporal distribution of RSPM in the study area were presented in **Figures 4(a)** and **(b)**.

**Table 9. Statistical summary of ambient air quality status of Burdwan municipality during pre and postmonsoon season.**

Statistics	RSPM ( $\mu\text{g}/\text{m}^3$ )		SO <sub>2</sub> ( $\mu\text{g}/\text{m}^3$ )		NO <sub>2</sub> ( $\mu\text{g}/\text{m}^3$ )	
	Premonsoon	Postmonsoon	Premonsoon	Postmonsoon	Premonsoon	Postmonsoon
Average	188.56	53.03	5.12	8.51	92.51	162.85
Maximum	363.6	141	26.69	23.11	238.19	937.25
Minimum	54.45	4.48	0.34	BDL*	3.79	23.91
Standard deviation	88.63	38.27	6.27	7.11	64.78	184.80

\*BDL indicated below detection limits

**Figure 4. Digital Elevation Model (DEM) on spatio-temporal distribution of RSPM over the study area in (a) Premonsoon (b) Postmonsoon.**

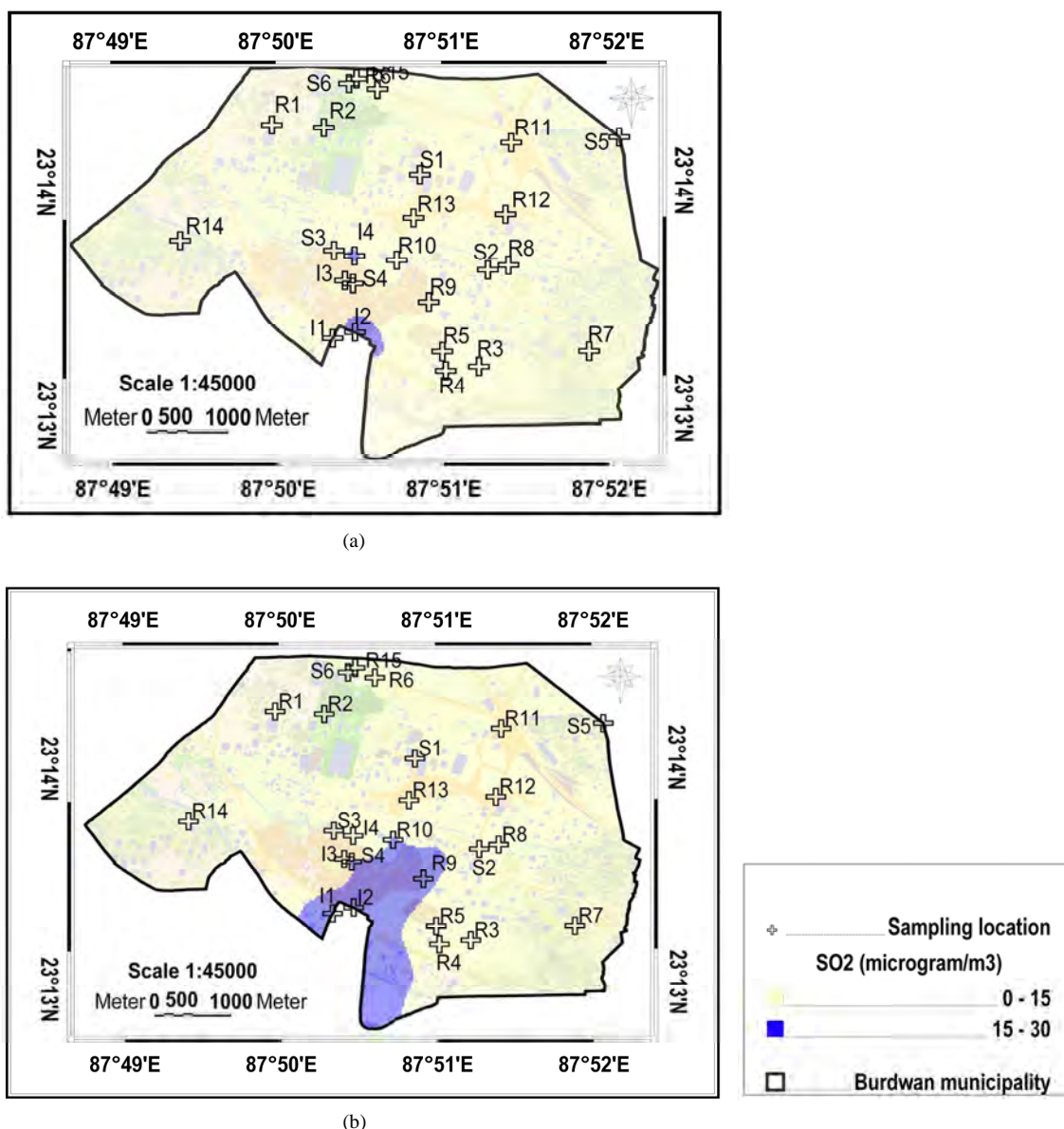


Figure 5. Digital Elevation Model (DEM) on spatio-temporal distribution of SO<sub>2</sub> over the study area in (a) Premonsoon (b) Postmonsoon.

### 3.2. SO<sub>2</sub> Scenario

The concentration of SO<sub>2</sub> was comparatively lower in both the seasons than the prescribed standard of NAAQS in all the monitoring sites. Similar kind of SO<sub>2</sub> status was also highlighted by other research workers such as Reddy and Ruj 2003 [21] and Gupta *et al.* 2008 [8]. Among industrial, residential and sensitive sites maximum SO<sub>2</sub> level was observed in industrial sites *i.e.* 26.69 µg/m<sup>3</sup> during premonsoon and 23.11 µg/m<sup>3</sup> during postmonsoon. This might possibly be due to emission from industrial boiler, heating and cooking sources. Within in-

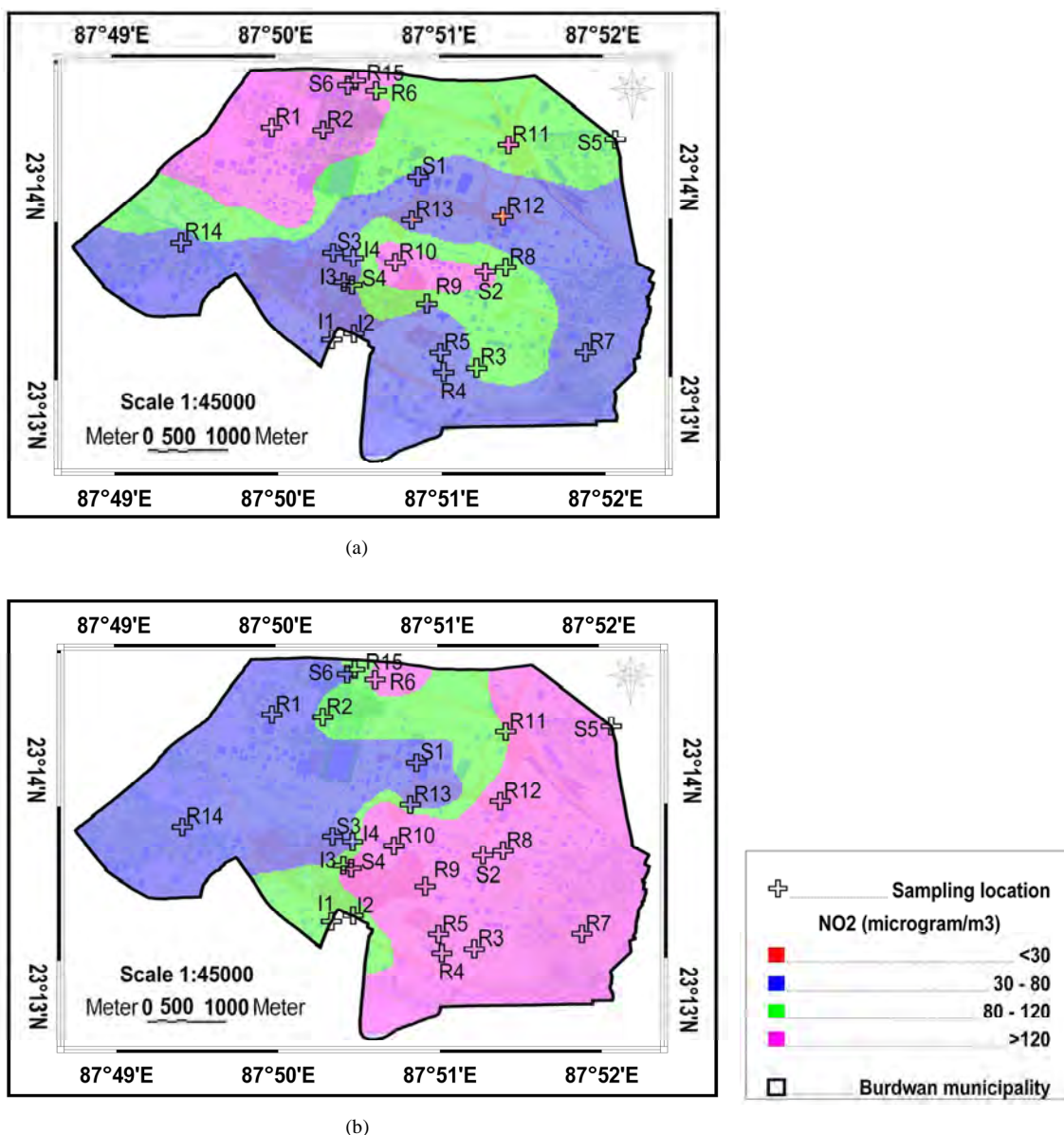
dustrial sites except I1, rest of the three sites had low SO<sub>2</sub> level during postmonsoon. While in case of residential sites most of the sites has higher level of SO<sub>2</sub> concentration during postmonsoon except R3, R11, R13, R14 sites. Burning of coal by local people might influence it. Similar trend also followed by most of the sensitive sites except S1 and S6.

Spatio-temporal distribution of SO<sub>2</sub> concentration are represented in **Figures 5(a) and (b)**.

### 3.3. NO<sub>2</sub> Scenario

Through out the study area NO<sub>2</sub> level was very high. Ma-





**Figure 6. Digital Elevation Model (DEM) on spatio-temporal distribution of NO<sub>2</sub> over the study area in (a) Pre-monsoon (b) Postmonsoon.**

ximum concentration was observed in S5 site in the tune of 937  $\mu\text{g}/\text{m}^3$ . This elevated level might be attributed to the high traffic density of the town. This was also supported by a published work of [33]. Among industrial sites, I2 and I4 show the low level of NO<sub>2</sub> concentration in postmonsoon while in the same season high concentration was observed in site I1, I13. To explain the later it could be said NO<sub>2</sub> was not only dependent on rainfall but also dependent on vehicle density and the distance of the monitoring site from road [18]. Among all industrial sites only site I1 had the higher-level of NO<sub>2</sub> than the standard in postmonsoon season. Regarding residential sites, R1,

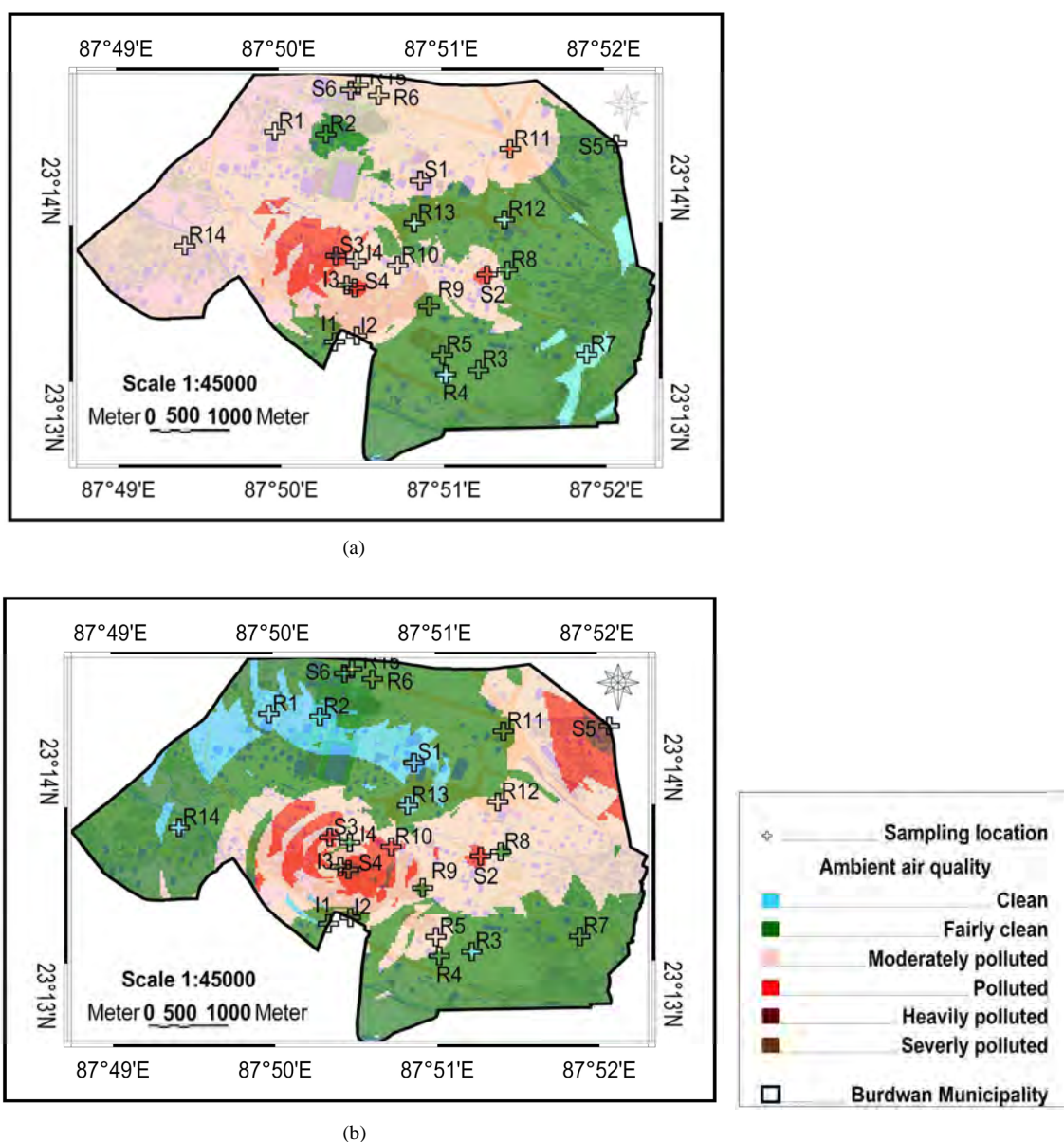
R2, R3, R6, R8, R10, R11, R15 had higher level of NO<sub>2</sub> concentration in premonsoon season while in postmonsoon except R1, R11, R13, R14 all have shown higher level of NO<sub>2</sub> than the prescribed standard. In the sensitive sites both the pre and postmonsoon value of NO<sub>2</sub> were exceeded its standard.

Digital elevation model with respect to spatio-temporal distribution of NO<sub>2</sub> were represented in **Figures 6(a)** and **(b)**.

### 3.4. Overall Scenario of RSPM, NO<sub>2</sub> and SO<sub>2</sub>

Average concentration level of all the pollutants in both





**Figure 7. Continuous surfaces from point data (AQI) by using Inverse Distance Interpolation (IDINT) technique in (a) Pre-monsoon (b) Postmonsoon.**

the season was represented in **Table 9**. In general RSPM level varied from 363 to 54  $\mu\text{g}/\text{m}^3$  with a mean of 188  $\mu\text{g}/\text{m}^3$  in premonsoon and from 141 to 4.48  $\mu\text{g}/\text{m}^3$  with a mean of 53.03  $\mu\text{g}/\text{m}^3$  during postmonsoon season.  $\text{NO}_2$  level varied from 3 to 238  $\mu\text{g}/\text{m}^3$  with a mean of 92  $\mu\text{g}/\text{m}^3$  and 23 to 937  $\mu\text{g}/\text{m}^3$  with a mean of 162  $\mu\text{g}/\text{m}^3$  respectively during premonsoon and postmonsoon season. Whereas  $\text{SO}_2$  level varied from 0.34 to 26  $\mu\text{g}/\text{m}^3$  with a mean of 5  $\mu\text{g}/\text{m}^3$  and BDL to 23  $\mu\text{g}/\text{m}^3$  with a mean of 8  $\mu\text{g}/\text{m}^3$  respectively during premonsoon and postmonsoon season. Finally, to compare the pre and the postmonsoonal value of  $\text{SO}_2$  and  $\text{NO}_2$  in this town it was found

that in most places the level of  $\text{SO}_2$  as well as  $\text{NO}_2$  was increased in postmonsoon season in spite of lowering down by rain. This might be explained by over crowded condition in the town of Burdwan, which was also the center of commercial activities in the district. The building structures were constructed literally wall to wall with very narrow streets separating one block from the other. Even the vehicular traffic was at most times bumper to bumper and sometimes at a stand still every time it rained. Hence, the increased amount of exhaust gases in the air negated the effect of the monsoon rains [32].

Statistical significance (student t-test) of seasonal vari-

ation of air quality parameters were carried out for the combined data of pre- and postmonsoon. Result of t-test for the combined data was given in **Table 10**. The Table value (critical value) at 48 degree of freedom was 1.68 for left-sided alternative hypothesis. Since the computed values of t were greater than the critical value of 1.68 for all the parameters the difference of means between pre and postmonsoon was significant at 5% level. Hence, the results clearly indicated that there was significant monsoonal effect on mean values of RSPM, SO<sub>2</sub> and NO<sub>2</sub>.

#### 4. Influence of Meteorological Parameters

In the study area premonsoon temperature ranged from 16 to 35°C while in postmonsoon it varied from 16 to 33°C. Humidity ranged from 42 to 75% and 53 to 85% during premonsoon and postmonsoon season respectively (**Table 4**). Regarding rainfall study area received an average rainfall of 78 mm in premonsoon where as in postmonsoon it receives 324 mm. Windrose which was graphical representation of wind data giving the % frequencies of wind speed, wind direction for a given location was represented in **Figures 3** and **4** for premonsoon and postmonsoon season respectively. From windrose diagrams it was found that the percentage of calm condition is higher (11%) in postmonsoon than premonsoon season (3%). As a result, higher concentration of gaseous pollutants was observed in postmonsoon than the premonsoon in the study area of this town. The wind speed ranged between 0 to 18 km/hr. Wind blew almost from all direction. But during premonsoon the predominant direction was from North-East, South-East and South-West direction while in postmonsoon season it was mainly from South-East, South-Southwest and North direction also. The major significant changes in the spatial and temporal variation of the ambient air quality of the town were due to variation of rainfall in the two seasons which was also supported by the **Table 10**.

#### 5. Season-Wise Classified Image on the Basis of Air Quality Index (AQI)

Air Quality Index of all the three categories of monitoring sites was represented in **Tables 6, 7** and **8**. From AQI status of the all monitoring sites in both pre- and postmonsoon season it was found that 40% of the total monitoring sites (I4, I2, I3, R1, R2, R3, R11, R14, S6, S1) became less polluted in postmonsoon. Whereas 40% of the total monitoring sites (I1, S4, S5, R4, R5, R7, R8, R10, R12, R15) became more polluted in postmonsoon season and 20% of the total monitoring sites (R6, R9, R13, S2, S3) remained same in status *i.e.* they are indifferent of rainy season and by using IDINT technique, season wise continuous surfaces of AQI have been gen-

**Table 10. Student t-test of mean difference between air quality parameters.**

Parameter	Calculated t value	Tabulated value of t at 0.05 level	Significant/Insignificant
RSPM	6.88	1.677	Significant
SO <sub>2</sub>	-1.7519	1.677	Significant
NO <sub>2</sub>	-1.7597	1.677	Significant

**Table 11. Season-wise percentage area of different AQI status.**

AQI status	Seasonal areal coverage (%)	
	Premonsoon	Postmonsoon
Clean	2.19	9.43
Fairly clean	41.09	52.02
Moderately polluted	53.37	30.18
Polluted	3.30	7.52
Heavily polluted	0.05	0.82
Severely polluted	Nil	0.03

erated. The output surfaces generated by IDINT were unclassified grey scale images. Though output surfaces were smooth but it is difficult to compare these surfaces on the basis of seasonal trend. On the basis of AQI rating, and using classification technique, these seasonal images had been classified (**Figures 7(a)** and **(b)**). Premonsoon classified image revealed that the western part of the town mainly covered the moderately polluted area while the eastern part of the town covers the fairly polluted region. But after the offset of monsoon just opposite scenario was observed. The moderately polluted region was seemed to be shifted to eastern part while the fairly clean part was shifted to western part. This phenomenon was also influenced by meteorological phenomenon which might be supported by wind rose diagram. It was observed that in premonsoon season wind mainly blew from North-East, South-East and South-West direction. It looked that the pollutants were seemed to be dispersed more in these direction from the polluted region. In postmonsoon just opposite picture was found. The predominant wind direction was South, South-West and North direction. So, the just opposite dispersion of pollutants had been occurred from the heavily polluted region. According to IDINT surface classification on the basis of AQI clean and fairly clean area had increased upto 7% and 2% respectively in comparison to premonsoon, whereas moderately polluted classified area decreased to 23% (**Table 11**).

## 6. Acknowledgements

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# Van Panchayats as an Effective Tool in Conserving Biodiversity at Local Level

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## ABSTRACT

Forest vegetation of a community managed forest was studied along four aspects. *Quercus leucotrichophora* and *Pinus roxburghii* was the dominant species on each of the two aspects. Across the aspects the total tree density ranged between 193 to 324.3 ind/ha, sapling density between 119 to 258.6 ind/ha and seedling density from 249.98 to 845 ind/ha. The shrub density varied from 199.99 to 406.32 ind/ha and herb density from 9466.66 to 52483.33 ind/ha. The total basal area varied from 0.06 to 7.15 m<sup>2</sup>/ha at eastern and north facing aspect for *Quercus leucotrichophora* and *Pinus roxburghii* respectively showing that the forest is in young stage. Species diversity value for tree layer varied from 0.21 to 1.23 while concentration of dominance value ranged from 0.56 to 0.94. It was noticed that with an increase in species diversity concentration of dominance value decreases indicating inverse relationship between diversity and dominance.

**Keywords:** Van Panchayat, Aspect, Diversity, Bhatkholi, Community Managed Forest

## 1. Introduction

The Central Himalaya, accounts for 8.68% of the total Indian Himalayan area (59436 km<sup>2</sup>) and harbours rich biodiversity due to geographical and geological peculiarities subtending a wide range of vegetation types [1]. The Himalayan biodiversity is severely threatened by natural and anthropogenic means. The various disturbances present in the area are eroding this rich biological diversity day by day and have led to the expansion of xerophytic conditions [2]. The majority of the population in the region is agricultural and pastoral. Forests present around the agricultural fields are highly degraded due to continuous anthropogenic disturbances. Villagers frequently graze their cattle in the adjoining forest which influence the pressure beside this accidental fire also the main cause for degradations the forest. The exploitive management practices and the biotic stress exerted by hill population in relation to oak species have encouraged the pine in various ways [3]. Much of the area now occupied by pine was originally under the potential natural vegetation of oaks [4]. Conversion of oak forests to pine is still proceeding on larger scale this trend may lead to ultimate disappearance of the oak forest from the region. A reversal of this trend requires a through evaluation of

current management practices including local people participation. The proportion of the old growth forests are being removed at a faster rate than young forests are being constituted, as a result the proportion of the middle aged forest is not stabilized, but is instead increasing [3]. In order to maintain such a structure of forests indefinitely, heavy subsidy in the form of cultural practices is required. Conservation of biological resources under community based conservation system is a key tool to lessen the depletion of biodiversity. Various programmes have been implemented, for the conservation of biological resources in the Indian Himalaya under the protected area network.

The active participation and involvement of local people either at community or individual level is essential towards conservation of the forest and other natural resources. The Van Panchayats represent one of the largest and most diverse experiments in devolved common property management ever developed [5]. Most of the Van Panchayat in Uttarakhand were initiated after formation of Van Panchayat Act in 1931, on degraded sites under the control of the State Revenue Department. The Van Panchayats have been sustainably managing their forests for decades without any financial support. According to recent estimates, there are about 12,089 Van

Panchayats managing an area of 544965 hectares. The area under each Van Panchayat ranges from a fraction of a hectare up to over 2000 hectares [6]. The Community forests managed in accordance with Van Panchayat Act is a hybrid of state ownership and community responsibility. In contrast to civil forests, community forests are not open forests. Access and use of forests is guided by rules elaborately designed and implemented by the communities. A major objective of Van Panchayat is to rejuvenate and manage patches of civil soyam forests for local use; it also prevents neighbouring villages from intruding into this zone, once formally demarcated as a Van Panchayat forest.

In the present study we have tried to access the importance of management practices of the Van Panchayat on conserving and regenerating forest under their control.

## 2. Materials and Method

The present study has been carried out in the Van Panchayat forest of Bhatkholi situated between 29°32.98'-29°34.32' N latitudes and 79°41.44'-79°43.2' E longitude of Lamgara Developmental Block of Almora District (Uttarakhand), where villagers put efforts to conserve the surrounding forest (approximately 10.12 ha). The basic climate pattern is governed by the monsoon rhythm. The annual rainfall varied from 832.0 mm to 921.9 mm, mean maximum temperature from 16.7°C to 32.6°C and the mean minimum temperature from 5.8°C to 19.5°C [7]. Rock types mainly comprises of schist, micaceous quartzite, plutonic bodies of granodiorites and granites [8]. The vegetation type mainly comprises Himalayan moist temperate oak forest, subtropical pine forest. The dominated tree species of the Van Panchayat are *Quercus leucotrichophora*, *Pinus roxburghii*, *Rhododendron arboreum*, and *Myrica esculenta*. Information regarding the vegetation was collected through questionnaires which will be distributed to the villagers of the Van Panchayats. The house holds were selected randomly on the basis of number of family members and categorized in to small (< 4), medium (5-9) and larger (> 10). Estimations also made by actual observations of the number of head loads removed daily, actual number of days in a year in which collection is done, number of hours and the frequency of collection by each family. Four aspects south west, east, north west, and north were identified with in each aspect trees were analysed by placing randomly 10,100 m<sup>2</sup> circular quadrats, the size and number of samples was determined following [9]. Sapling, seedling and shrub were studied in 10, 5 × 5 m<sup>2</sup> quadrats placed randomly. The vegetational data were calculated for density, frequency, abundance [10]. Importance value index for trees was determined as the sum of the relative density relative frequency, relative domi-

nance [11]. Individuals of the tree species were divided in to three classes, Trees were consider to be individual > 30 cm cbh (Circumference at breast height), Sapling 10-30 cm cbh and seedling < 10 cm cbh [3]. Species richness was determined following [12]. Species diversity was computed by using Shannon-Wiener's Index [13] and Concentration of dominance (CD) was calculated following [14].

## 3. Results

### 3.1. Tree Layer

The total tree density ranged between 193 to 324.3 ind/ha and total basal area ranged between 5.26 and 9.41 m<sup>2</sup>/ha among all the aspects (**Table 1**). The highest tree density was that of *Pinus roxburghii* (275 ind/ha) at north facing aspect, where the lowest tree density was that of *Rhododendron arboreum* and *Quercus leucotrichophora* (6.6 ind/ha each) at north west and eastern aspect respectively. The *Pinus roxburghii* was the most dominant species in term of the total basal area and IVI (7.15 m<sup>2</sup>/ha and 287.97%) at north and eastern aspect respectively. Species diversity value for trees varied from 0.21 to 1.23 at east and north west facing aspect where as the concentration of dominance value ranged from 0.56 to 0.94 on north west and eastern aspect.

### 3.2. Sapling Layer

The total sapling density ranges from 119 to 258.6 (ind/ha) at eastern and north west aspect. Across all the aspect the total sapling density of individual species ranged from 6.6 ind/ha to 173 ind/ha each at north west aspect for *Pinus roxburghii* and *Quercus leucotrichophora*. The most dominant species was *Pinus roxburghii* (IVI = 228.71%) at eastern aspect, however the total basal area was highest for the same species (0.49 m<sup>2</sup>/ha) at northern aspect (**Table 2**). Species diversity value for sapling layer varied from 0.75 to 1.32 which was highest on north west aspect and lowest on east where as the concentration of dominance value ranged from 0.45 to 0.66 at south west and eastern aspect (**Table 3**).

### 3.3. Seedling Layer

The total seedling density varied from 249.98 to 845 ind/ha at north and north west aspect respectively. The seedling density was highest for *Quercus leucotrichophora* at the north west aspect (626 ind/ha) and lowest on north facing aspect for *Quercus leucotrichophora* (16.66 ind/ha). Species diversity value for seedling layer varied from 1.06 to 1.64 on south west and north facing aspect respectively while the concentration of dominance value ranged from 0.09 to 0.58 on north and north west aspect respectively (**Table 3**).

**Table 1. Vegetational parameters for tree and sapling layers.**

Aspect	Species	Density (ind/ha)	Tree		Density (ind/ha)	Sapling	
			TBA (m <sup>2</sup> /ha)	IVI (%)		TBA (m <sup>2</sup> /ha)	IVI (%)
South West	<i>Quercus leucotrichophora</i>	153	4.13	219.84	86	0.34	160.32
	<i>Pinus roxburghii</i>	20	0.32	35.17	60	0.28	116.63
	<i>Myrica esculenta</i>	20	0.84	45	13	0.03	23.04
	<b>Total</b>	<b>193</b>	<b>5.29</b>	<b>300</b>	<b>159</b>	<b>0.658</b>	<b>299.99</b>
North West	<i>Quercus leucotrichophora</i>	173	4.33	182.59	173	0.6	192.16
	<i>Rhododendron arboreum</i>	6.6	0.86	21.08	53	0.18	42.63
	<i>Pinus roxburghii</i>	26	0.41	38.89	6.6	0.02	53.07
	<i>Myrica esculenta</i>	33	1.09	57.44	26	0.18	12.13
	<b>Total</b>	<b>238.6</b>	<b>6.69</b>	<b>300.00</b>	<b>258.6</b>	<b>0.98</b>	<b>299.99</b>
East	<i>Quercus leucotrichophora</i>	6.6	0.061	12.04	26	0.11	71.28
	<i>Pinus roxburghii</i>	200	5.2	287.97	93	0.34	228.71
	<b>Total</b>	<b>206.6</b>	<b>5.26</b>	<b>300</b>	<b>119</b>	<b>0.45</b>	<b>299.99</b>
North	<i>Quercus leucotrichophora</i>	8.3	0.15	11.31	40	0.1	68.52
	<i>Rhododendron arboreum</i>	16	0.74	27.1	-	-	-
	<i>Pinus roxburghii</i>	275	7.15	225.05	93	0.49	192.74
	<i>Myrica esculenta</i>	25	1.37	36.53	13	0.03	38.73
	<b>Total</b>	<b>324.3</b>	<b>9.41</b>	<b>300</b>	<b>146</b>	<b>0.62</b>	<b>299.99</b>

### 3.4. Shrub Layer

The shrub density is highest for *Pyracantha crenulata* (366.66 ind/ha) at north west aspect while lowest 6.66 ind/ha each for *Urtica dioica*, *Carex nubigena* and *Berberis asiatica* at south west, north west and east facing aspect respectively (Table 2). The Species diversity value was highest on north west facing aspect (0.63) and lowest on north west aspect (0.21), however the concentration of dominance value varied from 0.79 to 0.93 on north and east aspect respectively.

### 3.5. Herb Layer

The total herb density varied from 7866.66 to 52,483.33 ind/ha at east and northern aspect respectively. Among all the aspect *Apluda mutica* was the most abundant herb present on all the four aspect having highest density value 11333.34 ind/ha on north west aspect. The least density were shown by *Ocimum americanum* (66.67 ind/ha) on the north west aspect. The Species diversity value was highest on south west (2.01) and lowest on north west aspect (0.84), however the concentration of dominance value ranged from 0.39 to 0.69 on south west and

north west aspect respectively (Table 3).

### 3.6. Regeneration

The population structure of some dominant species is given in Figure 1. The seedlings and saplings of *Quercus leucotrichophora* were present on all the aspect. On eastern aspect number of *Quercus leucotrichophora* seedlings was higher where as saplings was higher on northern aspect. Trees of *Quercus leucotrichophora* were present in the younger girth classes (30-60 cm) indicating good regeneration status of this species while *Pinus roxburghii* saplings and seedlings were higher at south western aspect, similarly the majority of trees of this species was that of younger and in the girth class of 30-60 cm (Figure 1(c)). Conversion of seedlings to saplings and saplings to trees of *Quercus leucotrichophora* was satisfactory indicating well regeneration pattern and effective forest management.

## 4. Discussion

The value of total tree density (193 to 324.3 ind/ha) reported in the present study falls within the range values reported earlier by [6,15,16] for different central Hima-



**Table 2. Density (ind/ha) for seedling, shrub and herb layers.**

Species	Aspect			
Seedling	South West	North West	East	North
<i>Quercus leucotrichophora</i>	266	626	173	16.66
<i>Rhododendron arboreum</i>	-	20	-	33.33
<i>Myrica esculenta</i>	73	173	53	133.33
<i>Pinus roxburghii</i>	26	26	26	66.66
<b>Total</b>	<b>365</b>	<b>845</b>	<b>252</b>	<b>249.98</b>
Shrub				
<i>Prinsepia utilis</i>	13.33	-	-	-
<i>Pyracantha crenulata</i>	240	366.66	193.33	325
<i>Urtica dioica</i>	6.66	-	-	-
<i>Berberis asiatica</i>	-	20	6.66	33.33
<i>Rubus ellipticus</i>	-	-	-	8.33
<i>Carex nubigena</i>	-	6.66	-	-
<i>Pyrus pashia</i>	-	13	-	-
<b>Total</b>	<b>259.99</b>	<b>406.32</b>	<b>199.99</b>	<b>366.66</b>
Herb				
<i>Apluda mutica</i>	11200	11333.34	6933.34	6266.67
<i>Galium aparine</i>	800	-	-	266.68
<i>Themeda anathera</i>	2100	-	133.34	-
<i>Rosea brocera</i>	1000	-	-	-
<i>Setaria homonyma</i>	2200	-	-	-
<i>Hypericum elodeoides</i>	250	-	-	-
<i>Anaphalis contorta</i>	600	-	-	266.67
<i>Desmodium podocarpum</i>	800	200	666.67	533.34
<i>Anaphalis busua</i>	-	-	-	2266.68
<i>Oplismenus compositus</i>	-	2066.67	133.34	-
<i>Micromeria biflora</i>	-	133.34	-	-
<i>Ocimum americanum</i>	-	66.67	-	266.67
<i>Carex nubigena</i>	-	-	-	400
<b>Total</b>	<b>18,950</b>	<b>13,800</b>	<b>7,866.66</b>	<b>52,483.33</b>

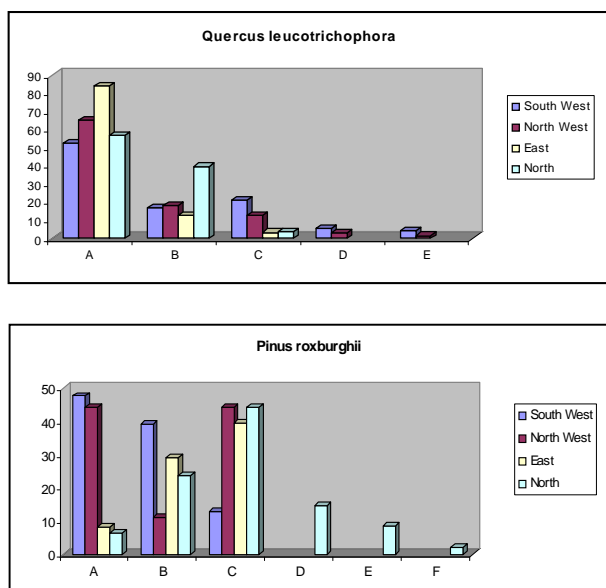
layan oak and pine forest (280-1680 ind/ha). The saplings and seedling density of *Quercus leucotrichophora* were higher on north western aspect where as eastern aspect shows least density. The lower density on eastern was due to encroachment of fuelwood, fodder and animal grazing by villagers. Conversion of seedlings to saplings

and saplings to trees of *Quercus leucotrichophora* was satisfactory indicating well regeneration pattern and effective forest management.

It is interesting that the previous research on chirpine indicates that pine is good reproducer not only in its own forest but also in other forest [9] and the replacement of

**Table 3. Species diversity (SD), species richness (SR) and concentration of dominance (CD) along different aspects.**

Aspect	Parameter	Vegetation layer				
		Tree	Sapling	Seedling	Shrub	Herb
South West	SD	0.94	1.31	1.06	0.47	2.01
	CD	0.65	0.45	0.57	0.85	0.39
	SR	3	3	3	3	9
North West	SD	1.23	1.32	1.17	0.63	0.84
	CD	0.56	0.51	0.58	0.87	0.69
	SR	4	4	4	4	5
East	SD	0.21	0.75	1.19	0.21	1.21
	CD	0.94	0.66	0.53	0.93	0.57
	SR	2	2	3	2	4
North	SD	0.84	1.24	1.64	0.59	1.74
	CD	0.72	0.49	0.09	0.79	0.43
	SR	4	3	4	3	7

**Figure 1. Population structures of two dominant species at different aspects; the relative density is on y-axis and the girth classes on x-axis; A = Seedlings, B = Saplings, Trees- C = 30-60 cm, D = 61-90 cm, E = 91-120 cm.**

the oak forest by pine has become a common and ever-increasing phenomenon [3], however from this study it is clear that if the forests are management properly the banj-oak will not disappear in near future. The total basal area of the present study for tree layer varied from 5.26 and 9.41 m<sup>2</sup>/ha. This pattern of total basal area was similar to the pattern reported earlier by [9,17,18], (35.02 to 83.77 m<sup>2</sup>/ha) for different oak forests of Kumaun Himalaya. The lower total basal area of the present study shows that the forest is in young stage. The Species di-

versity of tree layer of the present study varied from 0.21 to 1.23 while concentration of dominance value ranged from 0.56 to 0.94. These values are generally comparable with the values reported earlier by different workers for temperate forests [16,19,20]. Tree species richness were higher in north and north west aspect where as seedling species richness were higher north west and north facing aspect respectively. Where as the herb richness were higher on south west. It is a well known fact that the altitude and aspect represents a complex gradient along which many environmental variables change concomitantly. The ecosystem functions, distribution and occurrence of species had been affected by human interventions [2]. Among human influence, commercial exploitation, agricultural requirements, forest fire, and grazing pressure are the important source of disturbance [21]. However if the forest are managed properly with the involvement of the local community condition of their forest has been improved and the forests are free from heavy anthropogenic disturbances which also helpful for mitigating climate change and carbon trading.

Once the people start realizing that the carbon of their forests is saleable they will be motivated to conserve them. Thus, management of natural resources by the community becomes more evident keeping in minds its utility value and benefits to the communities. Forests of Uttarakhand are huge natural resource that could be tapped to eradicate rural poverty. It could be managed in a way to contribute more significantly towards rural livelihoods than at present. It is not possible to conserve these forests for long without participation of the community this would enable them to save the carbon sinks of Uttarakhand.

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# The Combined Application of WTP and WTA in Contingent Valuation Methods

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## ABSTRACT

*The In most contingent valuation (CV) studies, WTP (Willingness to pay) and WTA (Willingness to accept) were often used separately, so protesting or no response or even babbling answers are increasing, and the debate persists over the reliability of CV in economic policy analysis. In order to improve the reliability of CV, WTP and WTA is used synchronously to estimate the restoration cost of Maqu grassland ecosystem. Data were partly from questionnaire survey, and partly from interviews and authorities. Before conclusions were derived, we assumed these data that came from interviews and authorities were right. The main result is: If we assumed that the degraded grassland of Maqu needs 10 years to be restored, and divided the restoring period into two stages, then the restoration cost was  $0.85 \times 10^8$  RMB per year in former 4 years,  $0.022 \times 10^8$  RMB per year in latter 6 years. The total cost of Maqu grassland restoration was  $3.62 \times 10^8$  RMB. For all the costs of restoration, WTA occupied 94% and WTP only occupied 6%, suggesting that local grassland degradation was mainly caused by overgrazing and that the overloading livestock must be eliminated in order to achieve restoration successfully. Our research also showed that combining WTP and WTA in contingent valuation is very useful in estimating the cost of environmental improvement projects. Of course, whether these results are right or not, further researches are needed in the future, especially for the actual number of livestock in Maqu grassland.*

**Keywords:** Contingent Valuation Method (CV), Restoring Cost, Willingness to Pay (WTP), Willingness to Accept (WTA), Anchored Payment Card (APC)

## 1. Introduction

CV is a survey-based value elicitation approach which queries responders in systematic ways to state their willingness to pay (WTP) or willing to accept (WTA) hypothetical changes in some marketed and non-marketed goods or services. It has been widely used by economists to determine numerous environmental amenities or damage values, wetland restoration, nature protection, reducing health risk, drainage basin, public policy, welfare analysis, culture goods and other fields. Economists have been using and refining the method for well over two decades [1-7]. The contingent valuation method has been used extensively and described and reviewed elsewhere [8]. One of the insistent tropes of the contingent valuation literature is that we constantly ask individuals to estimate both their WTA and their WTP. The individual's willingness to incur the proposed costs reveals information about the value placed on the environmental improvements, while the individual's willingness to accept

the income change reveals information about the compensating variation that they associate with the proposed environmental change. WTA is just a method of weighing opportunity costs. Individuals can judge how much money they would need compensation considering the market experience [9,10]. Literally thousands of CV studies have been done by the world. However, in most previous CV studies, the economic value or cost of the posed environmental improvements were only from individual' WTP, researchers have spent much less energy on understanding the WTA measures because people often like to use willingness to pay rather than willingness to accept questions and someone may regard the problem with WTA exhibits a substantial hypothetical bias [11,12]. For lack of understanding WTA, protesting or no response or even babbling answers are increasing, and the debate persists over the reliability of CV in economic policy analysis [13]. Therefore, some researchers recommended that in CVM survey we should consider synchronously WTP and WTA of responders for unbi-

ased in cost-benefit analyses [14]. In this paper, we will try to combine WTP and WTA synchronously to estimate the restoration cost of Maqu degraded grassland ecosystem.

## 2. Methods

### 2.1. Study Area

Maqu grassland ecosystem is the main body of the Maqu counties which is located on the eastern Qinghai-Tibet plateau, Gansu province, China ( $101^{\circ}$ - $101^{\circ}$  E,  $34^{\circ}$ - $35^{\circ}$  N) (**Figure 1**). The altitude ranges from 2900 m to 4000 m with an annual rainfall of 450-780 mm. The annual average temperature is  $1.8^{\circ}\text{C}$  with below  $-10.7^{\circ}\text{C}$  in January and  $11.7^{\circ}\text{C}$  in July, the highest temperature during the growth season can reach  $23.6^{\circ}\text{C}$  to  $28.9^{\circ}\text{C}$ , and there are, on average, 270 frost days annually. The grassland area is about  $0.0087 \times 10^8 \text{ hm}^2$  and 59.32% of it belongs to alpine meadow.

Maqu is composed of 7 villages and 1 town (sub-administrative unit) with 0.04 million inhabitants, 73.4% of them being Tibetans. The county could be classified as a pure grazing area with  $1.0^8 \times 10^8 \text{ RMB}$  animal husbandry income in 2004, accounted for 99% of the total agriculture income. Grassland of Maqu acts as local resource supplement and natural ecological barrier of Yellow River. Yellow River comes from Qinghai Province, then flows around Maqu about 433 kilometers and again enters into Qinghai. The water supplement is  $27 \times 10^8 \text{ m}^3/\text{s}$ , so it is well known as the 'water tower of plateau'.

However, under the consistent interference of natural and human factors, grassland degradation raised in recent

years with its equilibrium broken, structures altered, and functions handicapped [15,16]. As degradation occurred, the average flux of Yellow River has been down from  $478.8 \text{ m}^3/\text{s}$  to  $393.8 \text{ m}^3/\text{s}$ , and the enhancements in local living standards were also limited [17,18].

Facing with these issues, governments have taken some measures, such as rotation herding, deratization etc. [19]. However, almost all restoration projects lacked local inhabitants' participation, and were controlled by governments. For lack of participation, restoration was slow and inefficient [20]. Therefore, in order to achieve degraded grassland restoration successfully, local herders must be guided to participate. Based on Ma [21], two approaches are often employed together to restore degraded ecosystem. One is natural approach by ecosystem pressure reduced and its resilience capability promoted. Another is to restructure or rebuild it by human actions. To local herders of Maqu, reducing grassland pressure is to dwindle in numbers of livestock because overgrazing is the key factor that caused Maqu grassland degradation [22]. While for degraded grassland restructured or rebuilt, herders have to raise funds to guarantee costs for materials. To summarize degraded grassland restoration, two type costs must be considered. In this paper, we can regard WTA the least compensation for herders to willingness accept due to forgoing their livestock, and WTP as the largest willingness to pay for degraded grassland restoration, which can enhance ecologic processes and structures, preserve regional and historical context, and encourage sustainable cultural practices and uses [23].

### 2.2. Questionnaire Construction and Investigation

It is the first time for local herders, who have no experience in making a price in the simulated market, to touch CV, so they can hardly express their WTP or WTA directly. Based on Cuena [24], Anchored Payment Card (APC) was employed as eliciting tools of CV because it can avoid starting point bias and can establish the context in which the bids should be submitted. The bid value as well as its interval derived from the pilot survey with open-ended format.

#### 2.2.1. Open-Ended Survey

In order to gain bid value for APC, the open-ended format questionnaire was designed, and 150 questionnaires were completed. Among 150 questionnaires, one third of them were handed out to local officials who were very familiar with grassland degradation and restoration. Additional 100 questionnaires were completed by 50 herders who were near Maqu city, and by 50 graduates who came from the State Key Lab of Arid-Agriculture of Lanzhou University. The main part of the open-ended format questionnaire is:



**Figure 1. Study area geography.**

Assume that local government was preparing an integrated project to improve the grassland quality and mitigate conflict caused by grassland and livestock. The project needs herders to support if grassland restored like 20 years ago. There are two ways for herders to support, including payment and reducing the number of livestock. Which one would you like to take? If you choose the payment way, then answer question (1), else answer question (2).

1) In next 10 years, how much money would you pay for supporting the plan from your family income per year? \_\_\_RMB.

2) If the government wants to buy your livestock for mitigating the pressure on the grassland. Please could you tell me the compensation that would like to accept for reducing your livestock by one cattle or one sheep? \_\_\_RMB per cattle, \_\_\_RMB per sheep

### 2.2.2. Pilot Survey

In order to form the final survey that respondents understood and accepted the main description reasonably well, careful pilot survey was conducted by us as Arrow suggested [25]. Based on open-ended format survey, an APC format questionnaire was designed. Then, we did pilot survey through 50 local herders investigated with random sampling. Finally, according to recommendations given by some experts of Lanzhou University, bid value and its interval was adjusted moderately, and the formal questionnaire was shaped. Comparison formal questionnaire to open-ended format questionnaire, contents changed a little except closed or half-closed questions substitution for some open-ended questions, the main part of formal questionnaire is:

1) In next 10 years, how much money (RMB) would you pay for supporting the plan from your family income per year? Please choose the corresponding numerical value that described as below

0 5 10 20 30 50 100 150 200 300  
400 500 600 700 800 1000 1200 1500  
1800 2000 2500 3000

a) If your selection is zero, then the reason (s) is (are)

A With the limit of income

B Believing that government could raise enough from others

C The project may not get expected results

D Others

b) If your selection is not zero, then choose a corresponding payment tool

A restoring fee

B Donations

C Labour

D Others

2) According to two circs as described below, please choose the least compensation that you are willingness to accept separately

a) cattle per (RMB)

600 800 1000 1200 1500 2000 2500 3000  
3500 4000 4500 5000 5500 6000 6500 7000  
8000 9000 10000 15000 20000

b) sheep per (RMB)

250 300 350 400 450 500 550 600 650  
700 750 800 850 900 950 1000

### 2.3. Formal Investigation

Using randomized cluster sampling, we selected 100 households in each village and one town, then adopted an in-person investigation because it generally leads to the highest survey response and generally reduces the likelihood of sample selection bias and provides more control over the order. It also offers practical advantages in maintaining respondent motivation and allows the use of graphic supplements [13,25,26]. Six inquirers were trained before the formal investigation, including three graduates of Lanzhou University and three local translators who were teaching in local elementary schools.

The administration of the questionnaire took place during May 2005. Before interview, we gave each investigated household certain gifts as compensation for the possible inconvenience of being interviewed. In interview time, respondents were reminded of the available range of the budget constraints, and that their willingness to pay for the environmental program in question would reduce their expenditures for private good or other public goods [2,25]. There are seven villages and one town in Maqu Country, the planning sample was 800 households. However, survey was suspended for some reasons in august 2005 and only 559 questionnaires were completed. In order to avoid the estimated value wildly and implausibly, based on Arrow and Veiten [25,27], 19 questionnaires were eliminated for their WTP in excess of 2% of their income. Also, 13 questionnaires were eliminated for no response, and one was eliminated for its respondent under the age of 18. Besides these and the lost one, 525 questionnaires were valid, accounting for 94% of the total questionnaires.

## 3. Results

In many previous CV studies, only personal income was considered by researchers, and expenditure was always excluded, so information was insufficient in integrated economic factors analysis. Here, we brought expenditure into analysis in order to understand local economic state entirely and the economic factors related to WTP and WTA.

### 3.1. Socio-Economic Characters of the Respondents

The preliminary findings and summary statistics of the sample of 525 respondents were presented in **Table 1**,

**Table 1. The social characters of sample.**

Gender			Age			Education		
Male	489	93.1%	18-24	73	13.9%	Illiteracy	299	57%
			25-35	176	33.5%	Elementary	188	35.7%
Female	36	6.9%	36-50	185	35.2%	Junior	30	5.78%
			> 50	91	17.3%	Senior or above	7	1.3%

**Table 2** and **Table 3**. From **Table 1**, we can see that most of the respondents are males, revealing the fact that females are often busier and have little rights in local society, so even if they were at home, being shy and afraid of being reviled by their husbands for inglorious answers, they seldom talk to others, especially to strangers. The average age was 38.5, ranging from 75 to 18 years old. The age bracket can both reflect the opinions of the old generation and the willingness of the Contemporaries on grassland restoration. Generally, old herders have substantial experience in grassland management and have unique perspective on grassland improvement. Among 525 respondents, 299 were illiterate, and only 7 young respondents had junior and senior education experience. For lack of education, local stockbreeding is hard to be modernized. Due to little discrepancy in education, we eliminated it in WTP and WTA analysis.

Most herders tended to be antipathetic or even distorted the truth deliberately when we asked direct questions about their income and expenditure, so we adopted the indirect method to calculate it instead of inquiry it. For example, income was calculated by sales of livestock and its byproduct, and sales of medicinal herbs and so on. For the same reason, expenditure was estimated by tuition fee, medical care expenses, and prevention fee for livestock from illness as well as expenses of forage purchase for livestock to live through the winter. **Table 2** and **Table 3** showed that 31.1% of the respondents' expenditure was over their income, and 38.1% was equal to their income, and about 30% was lower than their income. Statistical analysis indicated that income has positive correlation with expenditure ( $r = 0.272$ ,  $p < 0.01$ ). The more herders earned, the more they spent.

### 3.1.1. The Restoring Approaches Selection

Among 525 households, nearly 75% (394 families) of them selected the WTP approach and only 24.6% (131 families) of them selected the WTA approach. The reason for this is that herders often judge the rich or the poor just by the number of livestock that one household has, so herders would not like to sell their livestock until livestock old enough [22]. While for herders who were richer comparatively (income was above 35000RMB), we found that they inclined to select the WTA approach

**Table 2. Income of the families investigated (unit: ten thousand RMB, %).**

Income	Percentage	Income	Percentage
0.5-1	27.1	3.5-4.5	9.3
1-1.5	20.6	4.5-5.5	5.2
1.5-2.5	12.3	> 5.5	9.7
2.5-3.5	15.8		

**Table 3. Expenditure of the families investigated (unit: ten thousand RMB, %).**

Expenditure	Percentage	Expenditure	Percentage
0.5-1	31.1	3.5-5.5	8.5
1-2.5	38.1	> 5.5	3.3
2.5-3.5	19		

(among 125 the rich, 38 of them selected the WTA approach). This can be explained as the more herders become rich, the more time they spend outside, and learned a few technologies for making money, so livestock was no longer as important as before and they liked to forge them. Statistical analysis indicated that age, sex, and education has no significant correlation with approach selection.

### 3.1.2. The Restoring Cost of WTP

The frequencies of bid value were shown in **Figure 2**. In calculation process, we did some regulations. For example, among bid value being less than 50, the frequency of 20 was the highest, so we took 20 as the representative value to be computed. For the same reason, 2000 was regarded as the representative value to be computed among bid value over 1000. The regulated bid value was detailed in **Table 4**. Using weighted mean method, the average WTP was computed and its value was equal to 359 RMB. There were 8000 households in Maqu country. As described above, about 75 percent households, namely, 6000 households were willingness to pay for grassland restoration, so the total WTP was  $0.022 \times 10^8$  RMB per year.



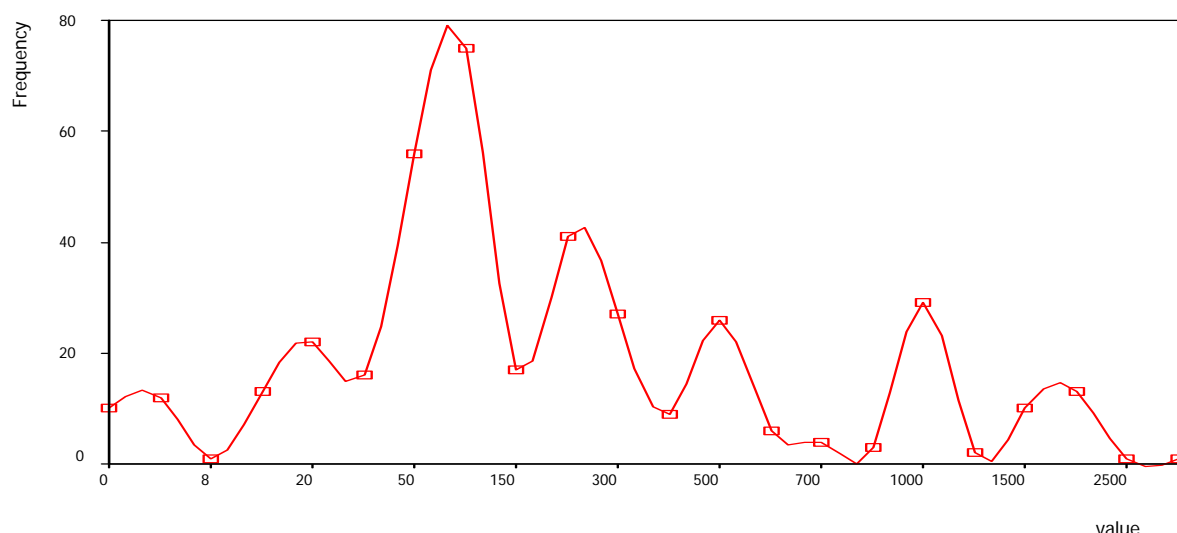


Figure 2. The frequencies of payment values (unit: RMB, %).

Table 4. The percentages of payment bids.

Bid	Percentage	Bid	Percentage	Bid	Percentage
20	5.6	200	10.4	700	1.0
< 50	18.8	300	6.9	800	0.8
50	14.2	400	2.3	1000	8.8
100	19	500	6.6	> 1000	6.9
150	4.3	600	1.5	2000	3.3

Among payment choosing, 34.7 percent of households selected pay for restoring fee, and 26.7 percent of households selected donation and 11.2 percent of them selected labor, and the remains selected the others.

The correlation analysis showed that income has weak correlation with WTP but not significant ( $r = 0.088$ ), and expenditure is not relevant to WTP and age has very weakly correction with WTP but not significant ( $r = -0.015$ ). Additionally, we used ordinary least squares regression. Several models were run using an enter process. The 'best' models gave adjusted  $R^2$  values of 0.005 for all bids case. These adjusted  $R^2$  values were very low, suggesting that there was not a good linear relationship between willingness to pay and the independent variables, so the regression equations were not useful for predicting a respondent's willingness to pay.

### 3.1.3. The Restoring Cost of WTA

Among cattle bid selections, 5.5 percent of the households selected the bid value that is over 10000 RMB. Obviously, herders wanted to affect the compensatory policy of the governments by high bid value, which was much more than the actual market value of cattle at that

time, so we eliminated them in analysis. While for sheep bid selections, we did not eliminate the high bid of 1000 for early reproductive age and short reproductive circle of ewes. Herders may consider the value of lambs in WTA bid value selecting process. After these unreasonable bids eliminated, the bid frequencies of cattle and sheep were shown in **Figure 3** and **Figure 4** respectively. The statistical results showed that the average cattle/sheep bid was 2545/660 RMB respectively. Both of the average values were very near but more than the actual market price at the time of the investigation.

The total WTA were derived from the overloading livestock in Maqu. According to the publicized data of local government, there were  $0.004 \times 10^8$  overloading sheep in Maqu grassland. But some officials said that the actual number of livestock was more than official figures because it is hard to check the amount of livestock for herders sparsely scattered and traffic inconveniences, and because some herders would not like to tell the truth. According to their experience, they considered that 20 sheep were not counted in each family, so there were  $0.0015 \times 10^8$  hidden overloading sheep in Maqu grassland. Comparison with the two data sets, we can think that the actual overloading sheep is  $0.005 \times 10^8$ . Of those surveyed, 24.6 percent of the households selected the WTA approach, suggesting that 2000 households would like to reduce their livestock. The average overloading sheep was 62.5 in each household, therefore, the total WTA was  $0.83 \times 10^8$  RMB per year.

The correlation analysis showed that the WTA of cattle has significant correlation with the WTA of sheep ( $r = 0.485$ ,  $p < 0.01$ ), this suggested that cattle and sheep have substitution relationship and herders could understand it

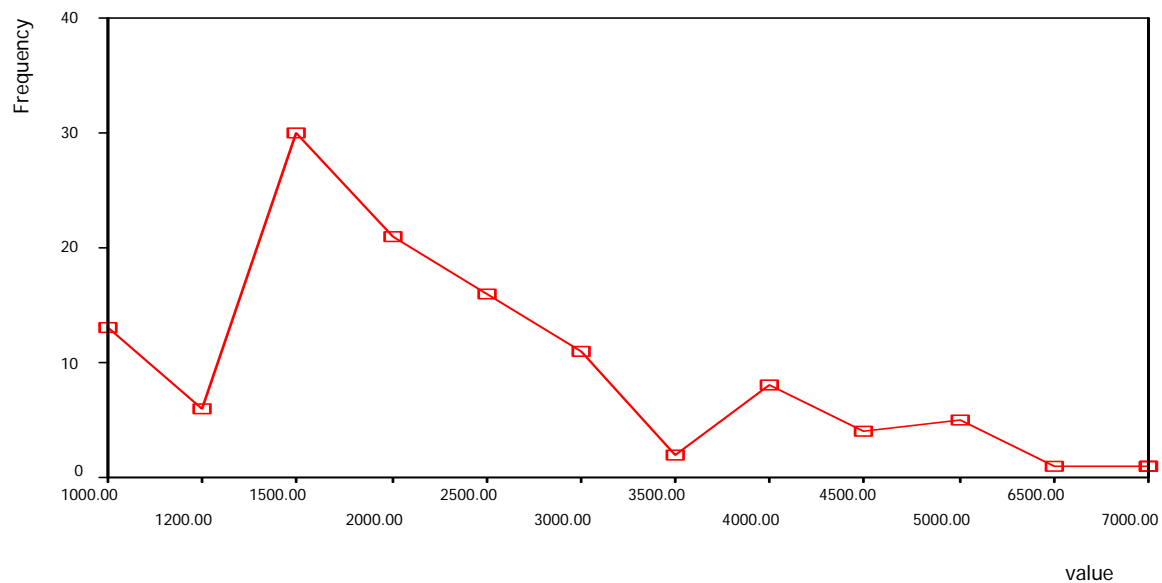


Figure 3. The frequency of cattle bid (unit: RMB).

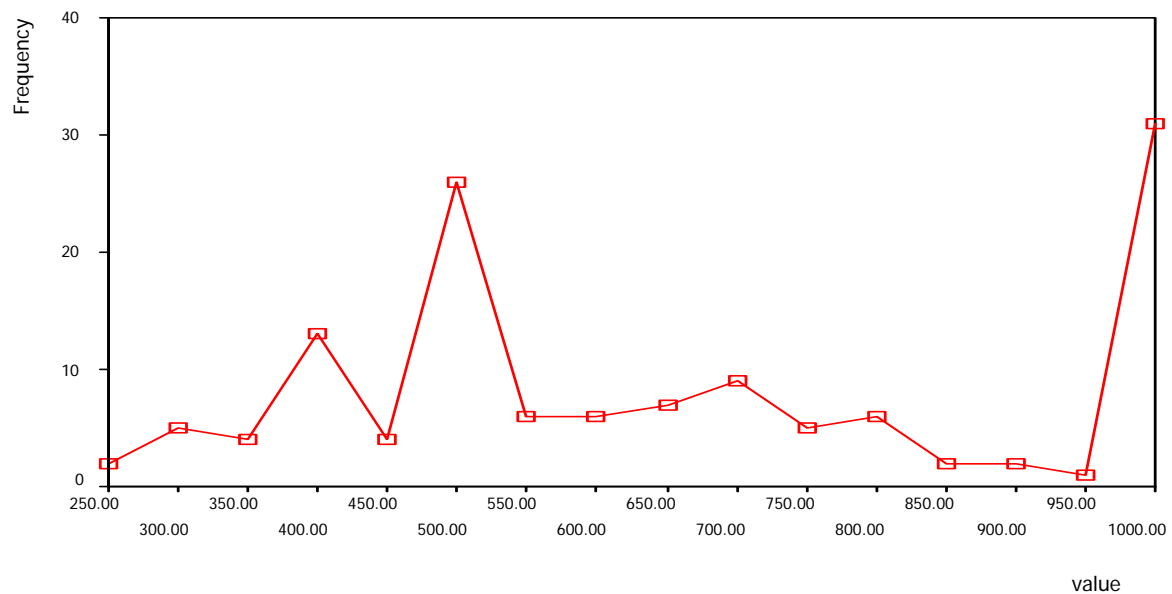


Figure 4. The frequency of sheep bid (unit: RMB).

entirely. The average bid of cattle (2545 RMB) was about three times the average bid of sheep (660 RMB). The result agreed well with the practical situation at that time, in which one cattle market price (2000 RMB) and was tripling of one sheep (600 RMB) and forage consumption per cattle was also three times per sheep. Finally, we tested the correlation of age, income and expenditure with WTA of sheep and cattle, and found no significant correlations. Furthermore, ordinary least squares regression analysis

showed that the regression equations were not useful for predicting herder's WTA for both cattle and sheep.

### 3.2. The Total Cost of Restoration

As described above, of those surveyed, 75.4 percent of households would like to pay for degraded grassland restoration, while 24.6 percent of households would like to accept compensation for forgoing their livestock to reduce stress of grassland and to help grassland to achieve

natural restoration, so the total cost of Maqu grassland restoration is the sum of WTP and WTA. If we assumed that  $0.0125 \times 10^8$  sheep (2000 households times 62.5) will be forgone per year by households, then the total overloading sheep would be eliminated within 4 years. Meanwhile, if we assumed that the degraded grassland of Maqu needs 10 years to be restored, and divided the restoring period into two stages, then the restoration cost was  $0.85 \times 10^8$  RMB per year in former 4 years, and was  $0.022 \times 10^8$  RMB per year in latter 6 years. The total cost of Maqu grassland restoration was  $3.62 \times 10^8$  RMB. For all the cost of restoration, WTA occupied 94% and WTP only occupied 6%, suggesting that local grassland degradation was mainly caused by overgrazing [22] and that the overloading livestock must be eliminated in order to achieve restoration successfully.

#### 4. Conclusions and Discussions

Ecosystem degradation is one of the most prominent global environmental problems. Therefore, to keep food safe and sustain a healthy environment, to prevent land from degrading, to restore and reconstruct degraded ecosystems, and to take effective actions to make the sustainable use of land resources becomes the hot spots of government organizations, non-government organizations, scientific communities, and the public alike. But in the field of restoration ecology, numerous articles address the ecological obstacles to restoring damaged ecosystems and strategies for overcoming these obstacles, little has been written regarding the question of how society to take strategies. The development of restoration ecology needs the cooperation of scientists, the government, and common people to restore the degraded ecosystem rapidly via the processes of exchanging information, methods, and experience [28,29]. CV as a social survey method, it provides a taking and communication platform to all kinds of groups that related to the environmental projects. By this platform, each group can obtain a lot of reliable information, enhancing the restoration projects process and efficiency. Based on our research, we can find that two ways can be adopted by local herders to take measures to overcome degradation. The first way is WTP way, namely, herders help degraded grassland to restore by willingness to pay for supplemental seeding and deratization and so on. The second way is WTA way, in which herders will be compensated for forgoing their livestock to reduce grazing press and to accelerate the natural restoration of degraded grassland. This is the first time for us to combine WTP and WTA in one questionnaire. From the results of research, at present, our suggestion for local degraded grassland restoration is that governments would use financial fund to buy the overloading livestock but not to fence. According to Gao [19],

from 1998 to 2002, the total cumulative cost of fencing ran up to  $3.9 \times 10^8$  RMB. The intention of fencing was to limit the number of livestock by grassland use rights assigned to individual households. A few years passed, the decrease in amount of livestock was little. For example, from 2000 to 2002, only 16190 livestock decreased. The average decrease was 5393 per year. If we regarded 5393 as the normal decrease in the future, then the total overloading livestock needs approximately 100 years or so to be eliminated. Comparing the cost between fence and restoration, it is very clear that government should use fencing fund to buy the overloading livestock, but not to fence continuously. Furthermore, government should make full use of the fund collected from the local herders to cooperate the natural restoration of the degraded grassland. This will shorten the period of restoration.

Another achievement resulting from our study is, in developing countries, that it is very necessary to compensate respondents during investigation although it is still a hot dispute topic in CV study. Based on Whittington [8], CV researches working in developing countries must determine what compensation is fair for the time spend in an interview. The compensation paid must be neither too low nor too high, but 'just right', 'just right' can be based on the minimum wage or the wages of an unskilled worker. However, as he pointed out, for the truly destitute in many developing countries, almost any payment could be perceived as coercive and thus deemed unethical. This may explain intuitively that the market economy and wage mechanism in developing countries is not mature yet so that researchers could not determine the right wage, so could the responders. From what he said it is inferable that CV researchers working in developing countries need not to compensate respondents. But based on our previous surveys, we found it is unwise for CV researchers not to carry out compensation because respondents may be not serious-minded and patient for free answering. In order to avoid this, compensation must be taken. Although we cannot determine the right compensation, we can determine the necessary compensation by customs, attitudes to time of locals and by the content of survey and so on. For example, in Maqu prides of herders would be hurt if we give money to them directly, so we decided to use gifts (candy, fruit, taking pictures) as compensation methods, and found that they inclined to accept these gifts. Therefore, compensation has no fix form, and CV researches can adopt various forms according to experience of themselves and culture and preferences of studied areas.

The issues in this paper are: during surveying, we just permitted respondents to select one approach from between the two options of WTP and WTA, and excluded those who want to take both, this may have little effects

on the reliability of the results. Some respondents said that they would like to pay 15 percent of the government restoration fund. From this, we can infer that some respondents may underestimate their real WTP in order to avoid additional payments when in WTP bid value selection process [30]. While other researchers said, respondents often overestimate their WTP [13]. The discrepancy between the stated WTP and the real WTP needs to test in practice [6]. In generally, for loss aversion or transaction costs, WTA is often high [31]. However, our study showed that respondents' WTA was very close to the market price during investigation time. Can this confirm that WTA is a very useful tool in valuing private goods or services that they have to forgo for environment improvement? Finally, handicap of communion between respondents and translators as well as others intervention when one were answering questions may affect the reliability of the results too. At the same time, the cost of WTA is based on the number of the overgrazing livestock. Therefore, if the number of livestock is accurate, then our results are right. On the contrary, if the number of livestock is wrong, then our results should be adjusted. In fact, there are many versions about the number of the livestock in Maqu, so our results would be used carefully before the actual number of livestock is known.

To sum up, this is the first time to use WTP and WTA simultaneously in CV studies. Although there are some defaults, it will continue to plow forward in choppy seas as Epstin said [9]. In order to improve our work, much should be done in the future.

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# Complex Processing of Pulverized Fly Ash by Dry Separation Methods

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## ABSTRACT

*Pulverized fly ash (PFA) is produced about 500 billions tons every year in the world in a result of coals combustion. Most of the fly ash collected in power plants is disposed by deposition in landfills, situated as a rule near big cities with well developed infrastructure and high cost of land. Moreover, the pollution of environment by fine solid wastes is inevitable and takes place in area of residing of a basic part of the population. The only solution is a complex processing of fine wastes with a production of value added materials. New conception of complex processing of PFA is proposed on the base of facilities of Electro-mass-classifier (EMC) and other techniques. The characterization of separated fractions was carried out by SEM and optic microscopy, XRD, laser diffraction, Mössbauer spectroscopy and other methods. A fine fraction of glass microspheres presents the main interest as filler in various materials.*

**Keywords:** Pulverized Fly Ash (PFA), Processing, Solid Wastes, Utilization, Dry Separation, Fly Ash Components, Glass Microspheres, Magnetospheres, Fillers, Electro-Mass-Classifier

## 1. Introduction

Coal fly ash is the particulate matter remaining after combustion of the carbonaceous component of coal. The residual, accessory minerals, predominantly clay, siliceous and iron minerals generally comprise the bulk of the ash. Fly ash was considered to be the sixth most abandoned mineral in the USA [1]. Usually less than 20% is reused commercially all over the world, predominantly in cement or as a fill material in construction [2]. The main cause of low utilization of PFA is a polydisperse inhomogeneous mix containing remnants of unburnt carbon—coke or char. Other problem for processing of PFA is related to high fineness of this waste complicating the separation of carbon by dry physical methods. Such problem may be solved by the use a green engineering—Electro-mass-classifier (EMC) technique operating with charged aerosols in closed volume in a wide range of particle sizes. Acid fly ash of type F according to ASTM prevails among different coal combustion products. In addition, commercial potential of this PFA after separation is maximal [2]. So, the perspectives of PFA complex processing technology based on the dry separation methods for environmental protection and

profitable production of value added materials are better to show in such class of waste. The possible solutions for utilization with profit for other types of PFA—fly ash C according to ASTM classification and ash from low temperature boilers with fluidized bed were found as well. Based on the mechanical activation in EMC, these solutions are presented in our book [2].

## 2. Materials and Methods

Various specimens of PFA were used for investigations produced in the largest power plants with high temperature boilers of the former USSR – Reftinskaya GRES (specimen No 1, coal from Ekibastuz deposit), Troitzkaya TEC (specimen No 2, coals from Ekibastuz and Kuznetsk deposits, both plants from Ural region), Novosibirskaya TEC 5 (specimen No 3, coal from Kuznetsk deposit, Siberian region). The fineness of PFA specimens was close one to another due to use of similar electrostatic precipitators. Ash particles were ranged from 200 nm to 500  $\mu$ m with mean particles size about 40  $\mu$ m.

Different techniques were used for dry physical separation of PFA including Electro-mass-classifier (EMC) [3,4], magnetic separator with permanent magnet from

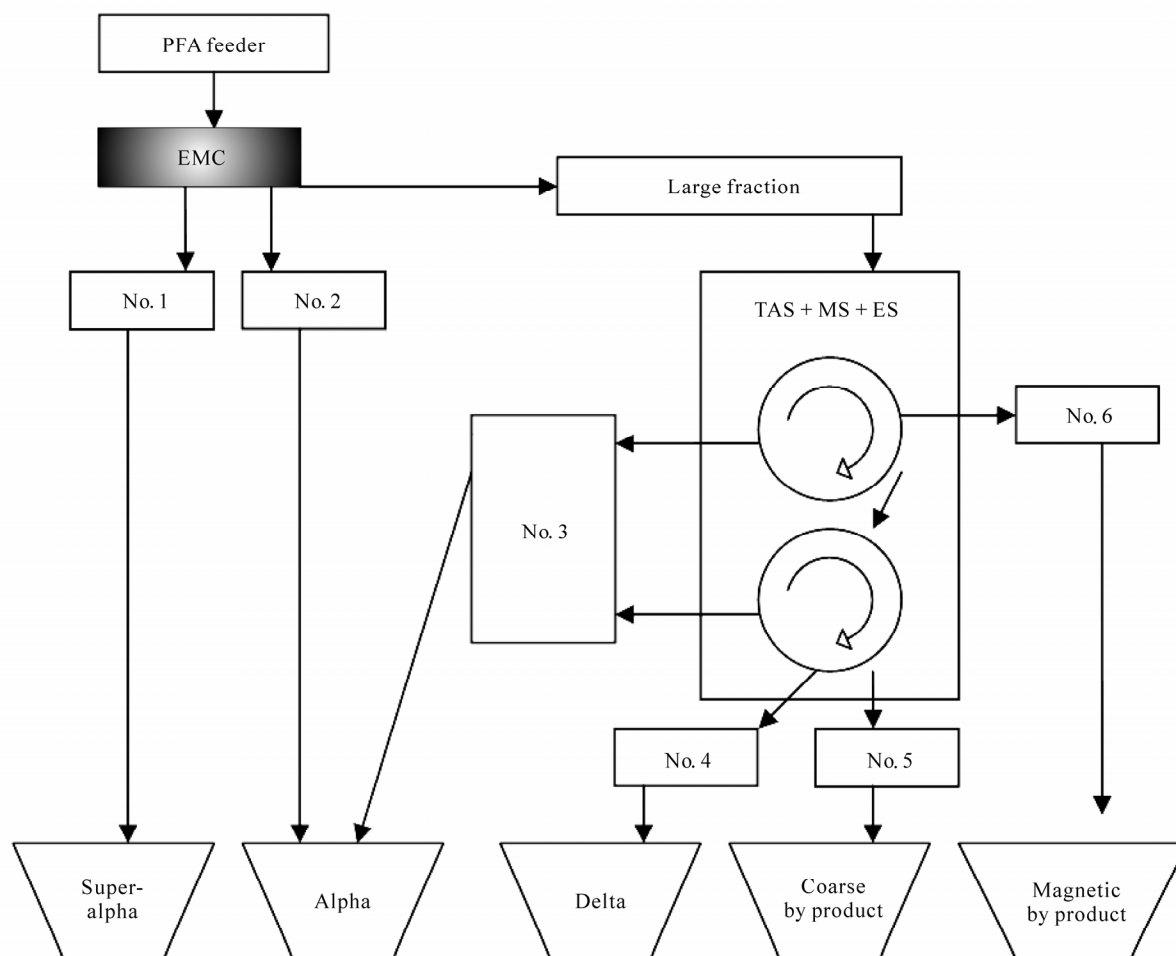
FeNdB-alloy (MS), triboadhesion separator (TAS), electrostatic separator with electric field 6 kV/cm (ES) and their combinations. All these devices are able to operate in discrete and continuous modes that are important for laboratory studies and industrial processing respectively. Moreover, all these installations are compatible and can operate in closed volume without aerosol contamination of air atmosphere. Flow sheet for processing of PFA may be realized for one run in production module with capacity 0.5-1 TPH. Operating in the range of particles size ~50 nm-2 mm, EMC technique allows to use with high efficiency other more selective separators after removing from PFA of the dust with particles size < 20  $\mu\text{m}$ . Principle of operation of multifunctional green EMC engineering is based on the generation of charged aerosols and their separation to unlimited number of fractions under action of centrifugal and other forth's [3,4].

The combination of instrumental and chemical approaches was used for characterization of separated ash fractions: XRD (DRON-3M, Russia, Bruker D8 Advance,

Germany, Cu  $K\alpha$  radiation), SEM + EDS (Hitachi TM-1000, JSM-6700F), optical microscopy Neophot 21, Germany, particle size analyzer (Laska, Russia), based on a laser diffraction method, Mössbauer spectroscopy (NZ-640, Hungary). Losses on ignition (LOI) were determined at 850°C, 1 hour. Chemical compositions were determined in spectrometer ARL-9900XP, Swiss.

### 3. Separation

In a result of investigation of dozen various PFA specimens of F type, the optimal scheme for complex processing was found, **Figure 1** [2]. After removing of fine and partly medium fractions in EMC, the rest of PFA can be divided by custom separators. Additionally four fractions can be obtained [2]. From practical point of view, fractions were called as potential products: Super-alpha (fine fraction No 1 in **Figure 1**), Alpha (medium fraction, No 2 + No 3), Delta (large fraction, No 4), coarse by-product (No 5), magnetic byproduct (magnetic fraction, No 6). Particle size distributions for obtained products



**Figure 1.** Flow sheet for complex dry processing of F type PFA.



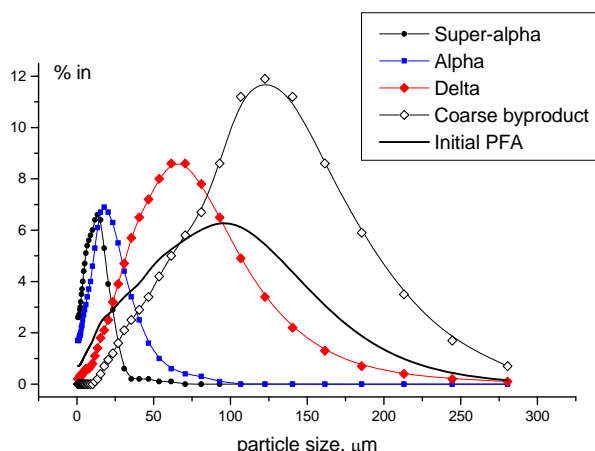
are shown in **Figure 2** for specimen No 1 (No 2 and 3 are very similar). Magnetic fraction has a particles size distribution close to Delta. After refinement of magnetic byproduct, magnetospheres with promising catalytic properties in methane conversion process can be received [5]. Magnetospheres present spherical particles of nanocomposites consisted from spinel and hematite in iron-rich silica glass matrix [5,6]. Coarse byproduct is a concentrate of relatively expensive materials—coke and cenospheres (hollow glass microspheres with density  $< 1 \text{ g/cm}^3$ ). Dry physical methods are not efficient for more deep separation of coarse fraction. So, the yield of this byproduct must be minimized to decrease the role of wet technologies in complete processing of PFA [2]. Fractions No 2 and 3 have close fineness and properties. So, for simplification of flow sheet these fractions are reasonable for uniting in a product Alpha. The yields of products and their LOI are presented in **Table 1**. For enrichment of coarse fraction by coke, relatively low electric field with  $E = 6 \text{ kV/cm}$  was applied in ES.

The carbon content in fine fraction hardly is higher than in initial PFA due to milling of porous char particles (**Figure 3**) during treatment in EMC in discrete mode of operation. The decrease of velocity of rotor rotation in EMC results to lowering of LOI in fine fraction. In EMC with continuous mode of operation, the life time of ash particles in camera of charged aerosol generation sharply drops resulting to a little milling of coke and lowered contents of carbon in fine and medium fractions—Super-alpha and Alpha respectively.

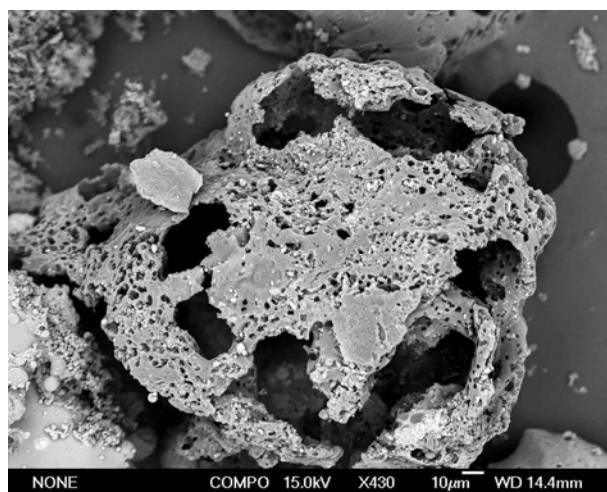
The ratio of milled carbon particles and capacity of EMC installations in separation process are linked parameters. From the other side, the pozzolana properties of Super-alpha product don't depend on the LOI. Moreover, after coke combustion at  $850^\circ\text{C}$ , activity of Super-alpha even decreases a little. There is a simple explanation of such effects. Fine carbon particles with a size  $\sim n \text{ }\mu\text{m}$  after milling get in pore space of cement stone with a little influence on the strength. In quenched ash glass particles mechanical strains results to high chemical activity in basic solution in mortar (so called mechanochemical activity). After thermal annealing, chemical activity decreases due to relaxation of mechanical strains in glass particles. Weak effects of carbon and strain relaxation on the strength of mortar with additives of Super-alpha show counterly signs and compensate each other.

#### 4. Chemical Composition

Chemical compositions of obtained products are presented in **Table 2**. These results are very similar to other known data [2]. The iron content in Delta is lowered



**Figure 2.** Particle size distributions for obtained products from PFA No 1.



**Figure 3.** Porous coke particle from PFA No 1.

relatively Alpha due to magnetic separation of large fraction only. Nevertheless, in EMC there is a facility to remove magnetic particles from fine fractions as well, if this operation has practical sense for better commercialization.

#### 5. Phase Composition

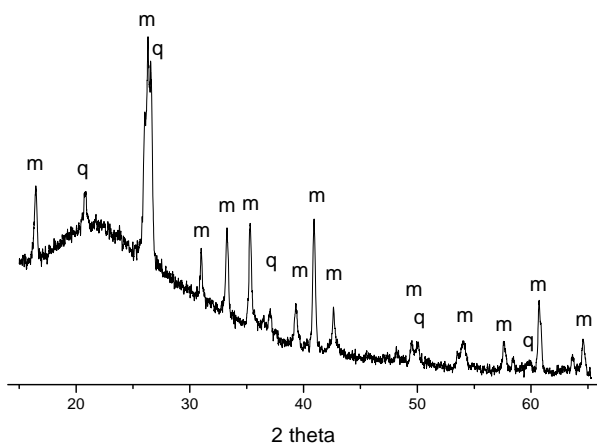
Phase compositions of all studied PFA specimens are very similar—small crystallites of mullite (m) and quartz (q) in silica glass, **Figure 4**. The content of mullite depends on the particle size. Fine fractions are enriched by quartz, and coarse fractions by mullite that correlates with chemical composition. Cenospheres are usually enriched by silica and Na/K, so the mullite content is lowered relatively main part of microspheres with density  $> 1 \text{ g/cm}^3$ . Phase compositions of magnetic fractions differ slightly as well, **Figure 5**. The main crystal phase is fer-

**Table 1. Yields and LOI (%) of products obtained in flow sheet in Figure 1.**

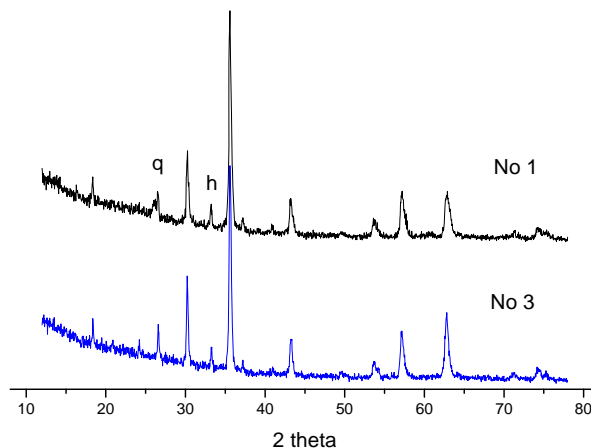
PFA, No	LOI	Products									
		Super-alpha		Alpha		Delta		Coarse byproduct		Magnetic byproduct	
		Y	LOI	Y	LOI	Y	LOI	Y	LOI	Y	LOI
1	2.8	9	3.6	45	2.1	36	2.7	7	4.9	3	1.0
2	3.5	8	4.4	39	2.4	39	3.0	12	8.4	2	1.0
3	3.9	7	4.5	35	2.9	45	3.2	10	10.9	3	1.2

**Table 2. Chemical composition of obtained products (for magnetospheres after refinement).**

PFA	Product	SiO <sub>2</sub>	Al <sub>2</sub> O <sub>3</sub>	Fe <sub>2</sub> O <sub>3</sub>	CaO	MgO	TiO <sub>2</sub>	K <sub>2</sub> O	Na <sub>2</sub> O	P <sub>2</sub> O <sub>5</sub>	MnO
1	S-alpha	61.3	24.4	3.72	1.97	0.96	1.0	0.81	0.58	0.62	0.08
	Alpha	60.5	26.1	5.64	2.39	1.09	1.18	0.68	0.39	0.40	0.11
	Delta	58.9	29.5	2.18	2.54	0.99	1.29	0.61	0.27	0.41	0.06
	Magnetospheres	26.8	10.9	55.1	2.35	1.14	0.91	0.17	0.43	0.10	1.93
2	S-alpha	62.7	24.0	4.0	1.55	0.7	1.06	0.8	0.4	0.41	0.07
	Alpha	60.1	26.1	5.1	1.74	0.76	1.23	0.59	0.36	0.48	0.14
	Delta	59.2	28.5	3.5	1.87	0.69	1.42	0.45	0.27	0.44	0.1
	Magnetospheres	24.9	9.69	57.1	1.66	1.59	0.95	0.26	0.34	0.25	1.95
3	S-alpha	62.0	20.2	4.22	2.05	1.52	0.75	2.9	1.59	0.5	0.05
	Alpha	59.8	22.3	4.96	2.82	1.88	0.86	2.81	1.52	0.45	0.10
	Delta	57.9	23.8	4.15	3.49	1.69	0.99	2.67	1.33	0.36	0.06
	Magnetospheres	22.3	7.1	61.1	2.91	2.90	0.10	0.41	0.79	0.45	2.4

**Figure 4. XRD pattern for PFA No 1.**

rospinel, and minor phases are hematite (h) and quartz (q). The content of ferrosphenel depends on the magnetosphere size with maximum content in medium fractions.

**Figure 5. XRD patterns of magnetospheres.**

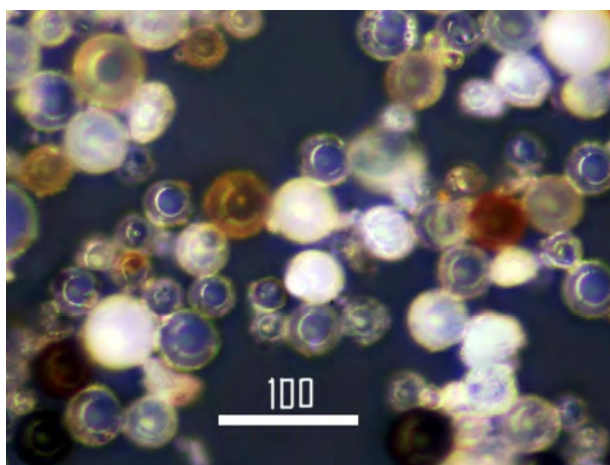
## 6. Morphology

Morphology of the most ash particles presents hollow

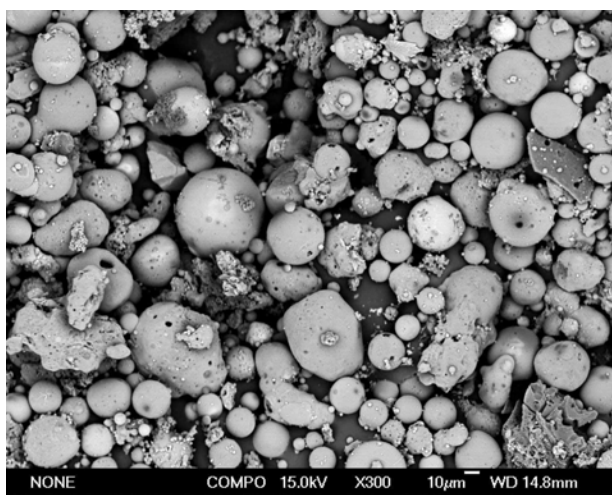
glass microspheres, **Figures 6-8** (marks in  $\mu\text{m}$ ). In large fractions some glass particles have irregular forms. In coarse fraction most particles are presented by agglomerates, including plerospheres and composites, **Figures 9-10**.

For many applications, spherical form of glass particles is very attracting, especially as filler in cement, concrete mixtures, polymer composites, and so on. So, the removing from PFA of coarse byproduct, consist of coke and nonspherical glass particles with irregular form and porous microstructure sharply enhances the quality of obtained products as fillers.

Part of carbon is in aggregates with silica glass microspheres, **Figure 10**. Because of this, the efficient removing of coke from PFA is limited by individual coke particles only. EDX analysis of glass particles in **Figure 9** displays a different chemical composition: for particle 1 -

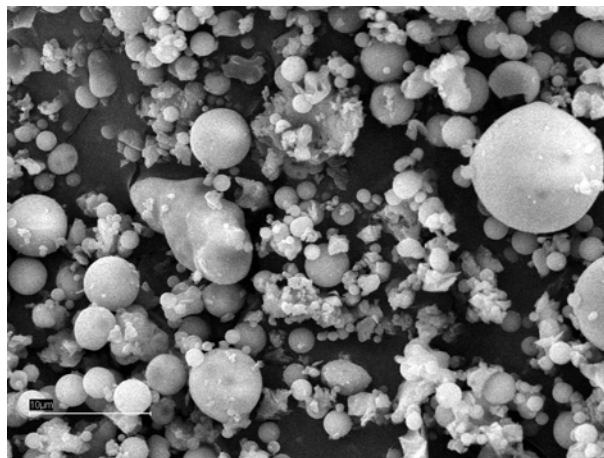


(a)

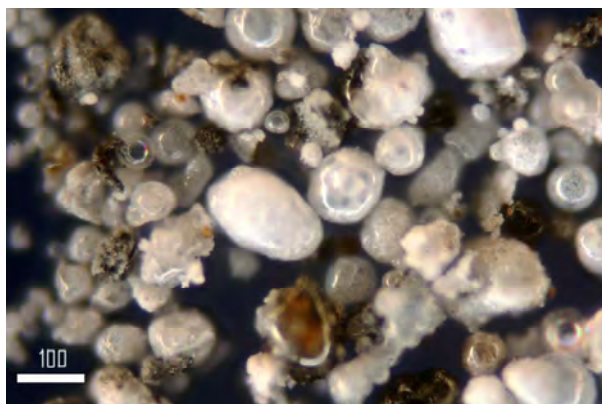


(b)

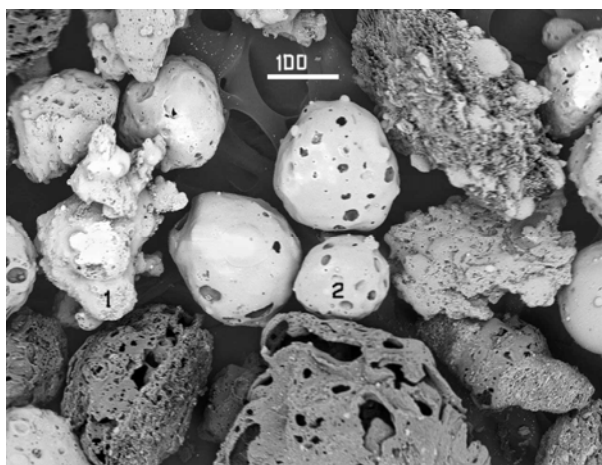
**Figure 6. Product Alpha. (a) optical image (PFA No 2); (b) SEM image (PFA No 1).**



**Figure 7. SEM image of Super-alpha No 1.**



**Figure 8. Optical image of Delta No 2.**

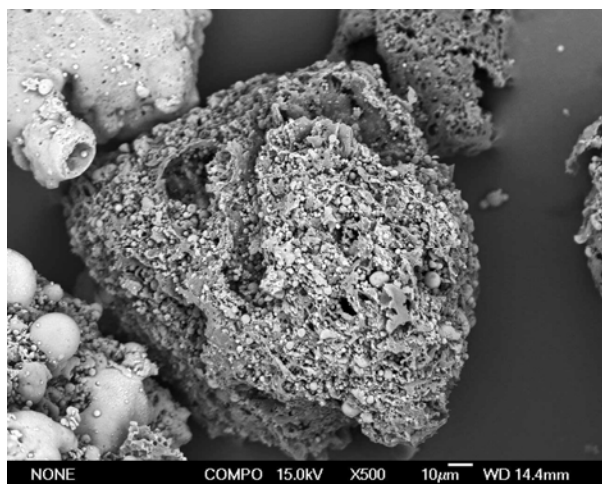


**Figure 9. SEM image of coarse byproduct No 1.**

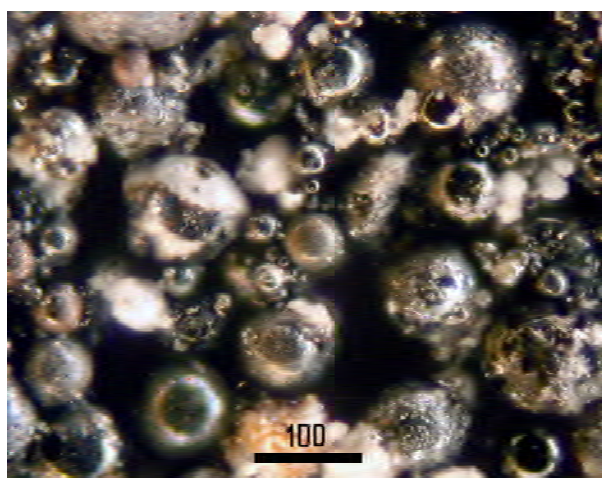
$\text{Si/Al} = 1.1$ , for particle 2 -  $\text{Si/Al} = 2.1$ , dark particles – coke aggregates.

Magnetospheres display various morphological types in every kinds of PFA, **Figures 11-13**. Magnetospheres from different power plants were studied by combination

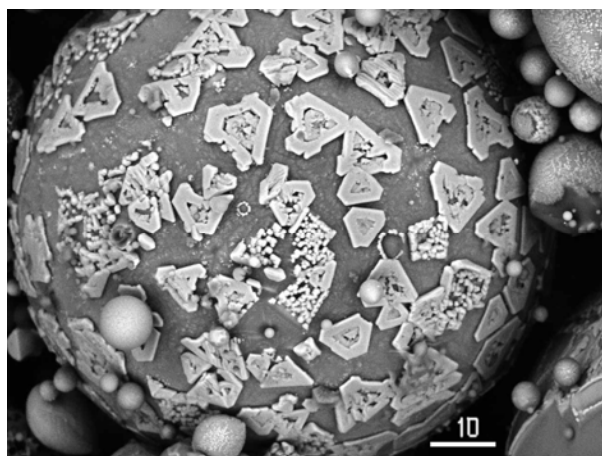




**Figure 10.** Aggregate from coke and glass microspheres in coarse byproduct No 1.

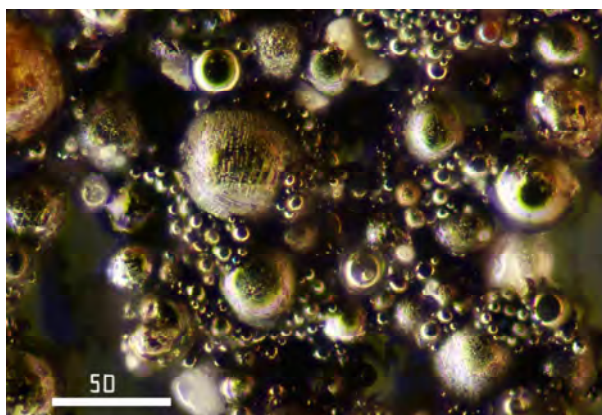


(a)

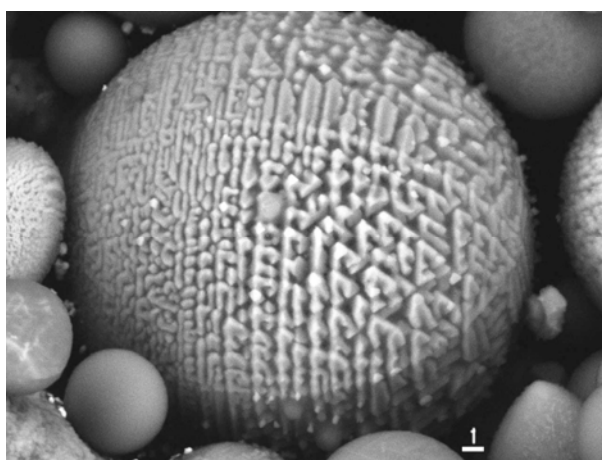


(b)

**Figure 11.** Optical (a) and SEM (b) images of magnetospheres No 1.



(a)



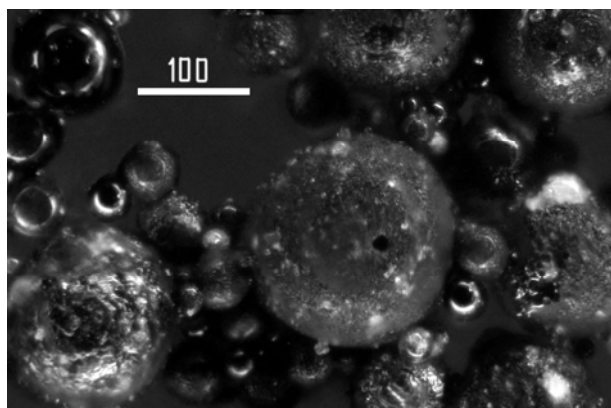
(b)

**Figure 12.** Optical (a) and SEM (b) images of magnetospheres No 2.

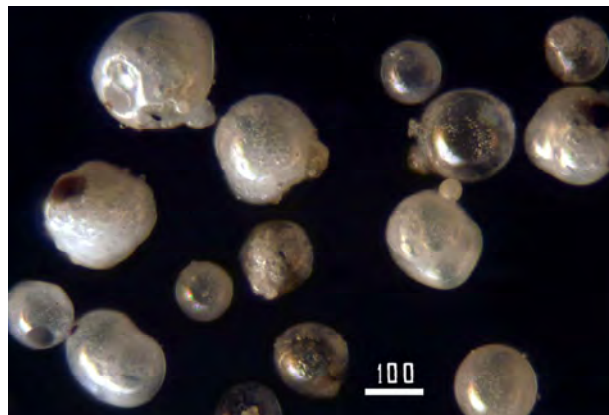
of structural methods—XRD with Rietveld analysis and Mössbauer spectroscopy [6]. Structural formulas of ferro-spinels were determined that allows to search a correlations with catalytic properties of magnetospheres with different origin [5]. Such correlations help to select optimal magnetospheres and modify them for promising applications in catalysis.

Cenospheres separated from coarse byproducts by wet technology are shown in **Figure 14**. Cenospheres are well known product with a relatively high cost and large area for applications [2]. The content of cenospheres in coarse byproduct is about 2-4% (up to 30% of total content in PFA).

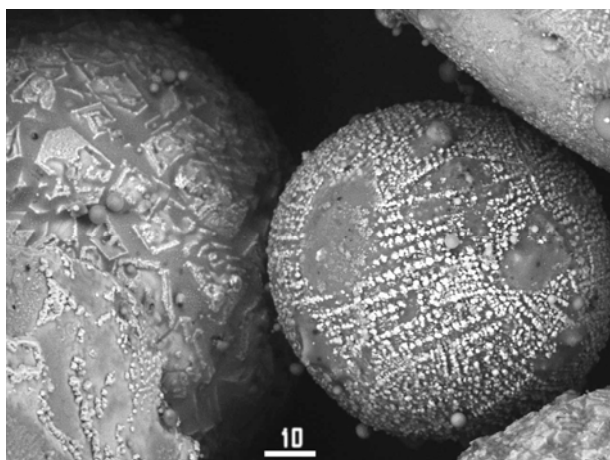
The iron contents in nonmagnetic fractions of glass microspheres and cenospheres in studied specimens of PFA are in the range 3-5%. As a result, the color of glass microspheres usually grey, that complicates their applications in white and colored polymer compositions. According to studies of glasses by Mössbauer spectroscopy



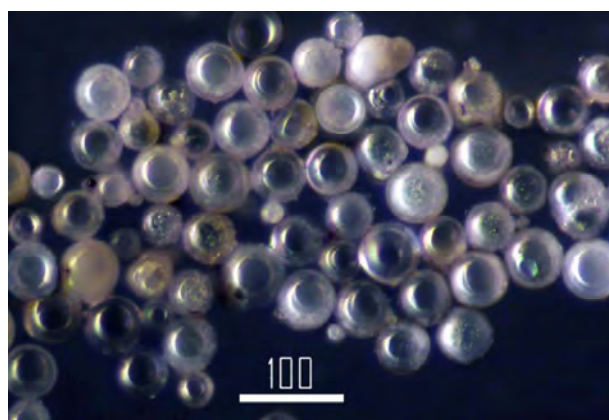
(a)



(a)



(b)



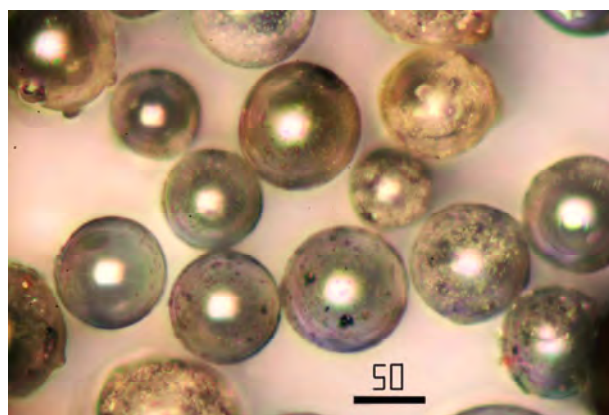
(b)

**Figure 13. Optical (a) and SEM (b) images of magnetospheres No 3.**

[2],  $\text{Fe}^{2+}$  state have coordination number 4.5 and  $\text{Fe}^{3+}$  - 6 (usual octahedral position). Parameters of iron in glass are presented in **Table 3**. After thermal treatment in air, the content of  $\text{Fe}^{2+}$  decreases with narrowing of lines that corresponds to annealing of mechanical strains in quenched glass. The color of powders acquires poorly red shade.

Other valuable product which can be separated from coarse byproduct is a coke with LOI > 90%. The content of extracted coke in coarse byproducts was about 2-4%. A coke represents a practical interest not only as a clean solid fuel, but mostly a source of rare volatile elements—V, In, Co, U (**Figure 16**), and as absorbent [2].

The obtaining of cenospheres and coke makes possible a profitable wet chemical processing of coarse byproduct. In such a case the waste after separation coke and cenospheres presents the source of rare earth elements, secondary products are silica, alumina and raw for zeolite synthesis [2]. Coarse byproduct may be used without



(c)

**Figure 14. Optical images of cenospheres divided from PFA No 1 (a), 2 (b) and 3 (c).**

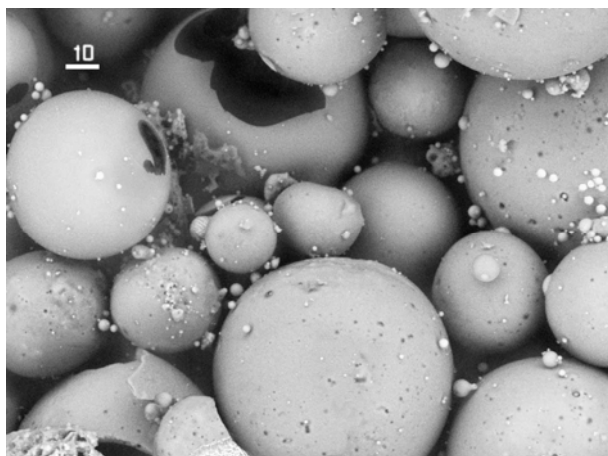
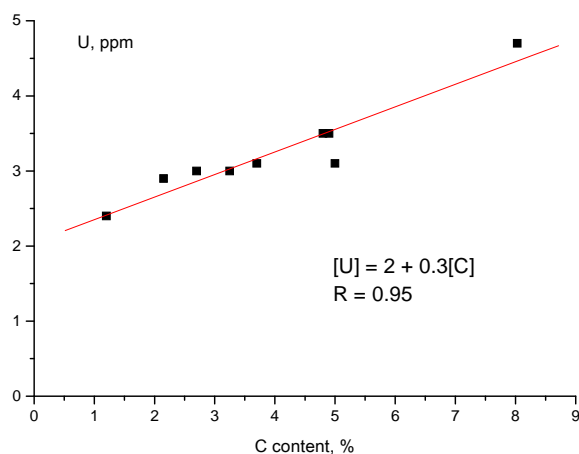
processing in fabrication of low dense building ceramics. However, the capacity of region market usually is less than quantity of produced coarse byproduct. Diversification of value added products allows to realize with profit a complete utilization of produced PFA.



**Table 3. Parameters of Mössbauer spectra of nonmagnetic cenospheres separated from PFA No 3.**

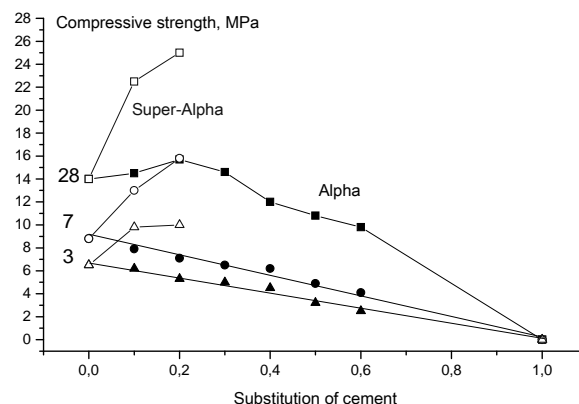
parameters	$\Gamma_1$	$\delta_1$	$\varepsilon_1$	content, %	$\Gamma_2$	$\delta_2$	$\varepsilon_2$	content, %
	Dublet $\text{Fe}^{3+}$				Dublet $\text{Fe}^{2+}$			
initial	0.868	0.333	1.154	61	0.778	1.010	1.945	39
$T = 850^\circ\text{C}$	0.838	0.322	0.991	88	0.560	1.007	2.088	12

$\Gamma$  - width of lines,  $\delta$  - chemical shift,  $\varepsilon$  - quadruplet splitting.

**Figure 15. Typical SEM images of cenospheres obtained from PFA No 2.****Figure 16. Correlation between U and carbon contents in fractions of PFA No 1.**

## 7. Pozzolana Properties

The utilization of PFA products is mostly as fillers in cement and concrete. The profitable processing of PFA is determined by the capacity of construction market. The quality of PFA products determines the possible volume of utilization. The pozzolana properties are shown in **Figure 17** for the fine PFA products.

**Figure 17. Compressive strength for mortars cement:sand = 1:3 with substitution of cement by products from PFA No 1 after 3, 7 and 28 days of curing in wet atmosphere.**

Partial substitution of cement by Super-alpha drastically increases the strength of mortars even after short time of curing. This enhancement of building material is related to increasing of density of concrete due to filling of pores between cement particles and absorption of excessive  $\text{Ca}(\text{OH})_2$ . The substitution of 30% cement by Alpha results to the same strength properties of concrete. The fineness of Alpha and custom cement are very similar, so the effect of substitution is related to pozzolana properties. A possible use of Delta in mortars or ready mix is due to partial substitution of sand. In this case a workability of mortars becomes better thanks to spherical form of particles, but the compressive strength displays a weak dependence on the degree of substitution. However, the density of concrete decreases, so normalized on the density effect becomes significant, **Table 4** [2]. The possible volume of PFA utilization in construction industry becomes much more significant after processing. Relatively low quality of mortars with initial PFA is related to remnants of coke and porous coarse ash particles with irregular form.

In the case of specially designed coal mix with inorganic additives, prepared with optimal morphology, the cost of PFA after combustion becomes more significant than produced electric and heat energy [7]. In other words, electric and heat energy must become byproduct

**Table 4. Parameters of 28 day concretes with products separated from PFA No 1, which are equal on the volume to mix cement:sand = 1:2 (weight ratio).**

No	Composition	Density $\rho$ , g/cm <sup>3</sup>	Compressive strength $\sigma$ , MPa	Normalized strength, $\sigma/\rho$
1	Cement: 2sand	2.43	36(3)	14.8
2	Cement: sand + Delta	2.09	32(3)	15.3
3	Cement: 2Delta	1.64	22(2)	13.4
4	Cement: 2magnetospheres	2.095	34.5(1.5)	16.5
5	(2/3cement + 1/3Alpha): 2sand	2.37	32(1.5)	13.5
6	(2/3cement + 1/3Alpha): sand + Delta	2.08	30(3)	14.4
7	(2/3cement + 1/3PFA): sand + PFA	2.09	24(1)	11.5

only in chemical reactors for the production of high temperature materials—glass microspheres and other fillers, binding materials, and so on.

## 8. Conclusions

PFA from coal power plants consist of components with a number of promising applications. One run dry physical separation in green EMC-based installation of PFA to 3 products and 2 byproducts provides wide possibilities for utilization with profit of solid wastes. Refinement of magnetic byproduct and wet processing of coarse byproduct can provide complete processing of solid wastes to value added products. In such a case, the effect of elimination environmental pollution is accompanied by economy of natural resources and lowering of CO<sub>2</sub> emission. Obtained results promotes to new eco-friendly conception to produce a power as a byproduct in relatively small chemical reactors which can provide better economic perspectives due to local green production of distributed energy from carbonaceous sources.

## 9. Acknowledgements

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# Sol-Gel Materials with Pesticide Delivery Properties

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## ABSTRACT

*Pesticides are widely used in agriculture, although they may create hazards both to humans and to the environment. In order to reduce the harmful effects of their administration, there has been made a great effort to find solutions. The porous sol-gel silica materials which are able to entrap different organic molecules represent new studied controlled release carriers. The aim of the present work was to prepare and characterize sol-gel composites based on trichlorfon as organophosphorous pesticide embedded in silica matrices generated from three different SiO<sub>2</sub> sources: tetraethylorthosilicate (TEOS), colloidal silica (CS), and sodium silicate (SS). Similar samples to those containing only trichlorfon have also been synthesised, in which  $\alpha$ -,  $\beta$ -, and  $\gamma$ -cyclodextrin have been included in order to study the possibility of improving the release of the pesticide from the silica matrices. The porous sol-gel silica materials generated from TEOS and CS are able to entrap the trichlorfon and ensure an efficient delivery of the pesticide. In the absence of cyclodextrins, better results are obtained in the case of TEOS precursor, compared to colloidal silica. The addition of cyclodextrins in order to improve the release of the pesticide from the silica matrices was successful only in the case of CS as SiO<sub>2</sub> precursor. The best release of the pesticide was obtained with  $\beta$ -CD.*

**Keywords:** Sol-Gel, Tetraethylorthosilicate, Colloidal Silica, Trichlorfon, Cyclodextrin

## 1. Introduction

Nowadays, agrochemicals play an important role in making possible the continuous increase of food production. On the other hand, a high level of chemicals could become a danger for humanity. Pesticides represent one of the most important types of chemicals used in agriculture. They include a wide diversity of substances. Among them, the class of organophosphorous compounds (OP<sub>s</sub>) has gained an enormous commercial success, due to their properties of controlling moths, ants, cockroaches, termites, fruit flies and similar insects, fleas, locusts, caterpillars and ticks [1]. They are the most frequently used in many cultures, at various stages of cultivation, as common insecticides, in order to control insect and arthropod pests on vegetable crops together with the increase of crop yields [2-8]. Among the organophosphorous pesticides, trichlorfon [O,O-dimethyl-(2, 2, 2-trichloro-1-hydroxy ethyl)-phosphonate] is one of the most popular, being ex-

tensively used in agriculture since 1952 as a broad spectrum insecticide [2,9-16]. One of the major problems of agriculture which must be solved refers to the controlled use of pesticides. The presence of the used pesticides in the surface and ground waters [4,6,17,18] emphasises this environmental issue. As a result of the increased awareness and worry regarding the potential of pesticides to have detrimental impacts on both human and ecosystem health, there is a great interest in developing less persistent but more selective pesticides. However, their use can be too expensive because they require more frequent application. Because of the high costs and the limitations in what the design of new pesticides is concerned, the industry and the researchers have decided not to fabricate new pesticides, but to improve the delivery systems of the current ones. An effective way is using the controlled release alternative. The controlled release systems have become more and more important due to their advantages: they minimize pesticide residues available to the envi-

ronment, thus reducing the environmental hazards and human toxicity, they increase the efficacy and the longevity of the pesticide and decrease the application costs, allowing less frequent uses [17,19-22]. Hussain [21] divides the controlled release formulations of pesticides into four main types: polymer membrane reservoirs, matrices containing physically-trapped pesticides, covalently-bounded pesticides and coated pesticide granule systems. Some examples are already mentioned in the literature [8,22-25]. As the property of the sol-gel process to prepare inorganic-organic hybrids by embedding organic molecules in the inorganic oxide matrix is a well known fact [26-35], Böttcher *et al.* [20] have used the sol-gel matrix as an alternative to biocide encapsulation. They prepared sol-gel composite films with controlled release properties.

The aim of the present work was to prepare and characterize sol-gel composites powders based on trichlorfon as organophosphorous pesticide embedded in silica matrices generated from three different  $\text{SiO}_2$  sources: tetraethylorthosilicate-TEOS, colloidal silica-CS, and sodium silicate-SS. Both routes of the sol-gel method (the alkoxide and the aqueous one) have been used. The aqueous route selection was based both on economical and ecological reasons: the aqueous  $\text{SiO}_2$  precursors are less expensive than TEOS and the soil attack is almost inexistent in a system in which the pesticide proceeds from an aqueous medium. Because the ability of cyclodextrins to form inclusion complexes with a wide variety of agricultural chemicals is well known [36-42], the possibility of using them in order to improve the release of the pesticide from the silica matrices has been studied. A comparison between the performances of the prepared materials has been made, in order to establish the most propitious reagents (e.g. silica precursor and type of cyclodextrin) and synthesis conditions which could lead to the best results from the point of view of the applicability.

## 2. Materials and Methods

### 2.1. Preparation of Samples

Composite samples, consisting of pesticide embedded in silica gels (pesticide/ $\text{SiO}_2$  = 1/3 in weight), have been prepared using both routes of the sol-gel method: the alkoxide and the colloidal one. The pesticide was an organophosphorous one, trichlorfon respectively: O,O-dimethyl-(2, 2, 2-trichloro-1-hydroxy ethyl)-phosphonate (T), from Jiangsu Hongze Chemical and Industry Co., Ltd. The  $\text{SiO}_2$  precursors were: tetraethylorthosilicate (TEOS) from Merck, colloidal silica Ludox SM-30 type (CS) from Aldrich, and a sodium silicate solution 26 wt.%  $\text{SiO}_2$  (SS) from Merck, respectively. In the case of TEOS, the ethanol (from Riedel de Haën) has been used as solvent. The gelation of all prepared sol-gel materials was ac-

complished at neutral pH, which is also totally harmless for the soil in case of application. The pH adjustment was realized with a 25 wt.%  $\text{HNO}_3$  solution. The experiments were made at the room temperature. All samples have been prepared under continuous stirring, in a few steps. First, the alkoxide was solved in the corresponding quantity of alcohol (the molar ratio of  $\text{SiO}_2/\text{ROH}$  = 0.075/0.515), respectively the colloidal silica and the sodium silicate solutions have been diluted with water, in order to ensure the same  $\text{SiO}_2$  concentration in all samples (solutions "a"). Then, the aqueous solution of trichlorfon (solution "b") was added drop by drop to solution "a", resulting solution "c". Its pH was checked and adjusted to the value of 6. For each of the obtained composite samples the corresponding  $\text{SiO}_2$  matrices have been prepared, in the same experimental conditions but in the absence of trichlorfon. In order to study the influence of the presence of cyclodextrins over the release of the pesticide from the prepared materials, similar samples to those containing only trichlorfon have been synthesised at the same time, in which  $\alpha$ -,  $\beta$ -, and  $\gamma$ -cyclodextrin (CD) from Wacker-Chemie GMBH have been included. All cyclodextrin types have been introduced in the reaction mixture as aqueous solutions, simultaneously with the trichlorfon, in the quantity imposed by the solubility limit. The composition of the corresponding samples was: 19 wt.% T, 24 wt.% CD and 57 wt.%  $\text{SiO}_2$ , respectively.

In the present work, a number of 15 gels have been prepared. Their names were established depending on the used  $\text{SiO}_2$  precursor, as follows: TEOS, CS, and SS (for the silica matrices); TEOS + T, CS + T, and SS + T (for the silica/trichlorfon composites); TEOS + T + CD ( $\alpha$ -,  $\beta$ -, or  $\gamma$ -), CS + T + CD ( $\alpha$ -,  $\beta$ -, or  $\gamma$ -) and SS + T + CD ( $\alpha$ -,  $\beta$ -, or  $\gamma$ -), respectively (for those composites in which both trichlorfon and cyclodextrin have been included in the silica matrices).

### 2.2. Methods of Characterization

The structural characterization of the prepared samples was accomplished by IR spectroscopy, using a FT-IR NICOLET 6700 ( $400\text{--}4000\text{ cm}^{-1}$ ) spectrophotometer. Their thermal behaviour was studied up to  $800^\circ\text{C}$ , using a Mettler Toledo Star System TGA/SDTA851/LF  $1600^\circ\text{C}$ , with a heating rate of  $10\text{ K min}^{-1}$ , dynamic air atmosphere, and a flow rate of  $50\text{ mL min}^{-1}$ .

The release of trichlorfon from the silica matrix was investigated during a series of batch experiments. In these tests, a quantity of sample corresponding to 100.0 mg trichlorfon (between 0.3863 g and 0.8803 g sample) was contacted with 100 mL ultrapure water and continuously mixed (150 rpm.) in a closed glass vessel at the temperature of  $21 \pm 1^\circ\text{C}$  using a thermostated shaker (GFL 3033). Trichlorfon concentrations from the aque-

ous solutions were determined by High Performance Liquid Chromatography (HPLC) technique, using a *Varian Pro Star* chromatograph equipped with an UV-Vis detector ( $\lambda = 200$  nm). A column Inertsil ODS2 (25 cm  $\times$  4.6 cm  $\times$  1/4") was used as stationary phase. The mobile phase was a mixture of acetonitrile/water (1:1, v/v) with a flow rate of 1 mL min<sup>-1</sup>.

### 3. Results and Discussion

In the experimental conditions mentioned above, 15 gels have been obtained after gelation times of hours (for the samples synthesized from TEOS as silica precursor), days (for the samples obtained from CS), and minutes (for the samples proceeding from SS), respectively. They have been dried at room temperature and subjected to characterization, as follows:

#### 3.1. IR Spectroscopy

All the prepared samples, and the organic compounds included in the sol-gel silica matrices (trichlorfon,  $\alpha$ -,  $\beta$ -, and  $\gamma$ -cyclodextrin) have been structurally characterized using the IR spectroscopy. **Figure 1** presents the IR spectra of the sol-gel prepared pesticide-silica composites in the absence of cyclodextrins in comparison with the corresponding spectra of the silica matrices that proceed from three different precursors and with the trichlorfon IR spectrum.

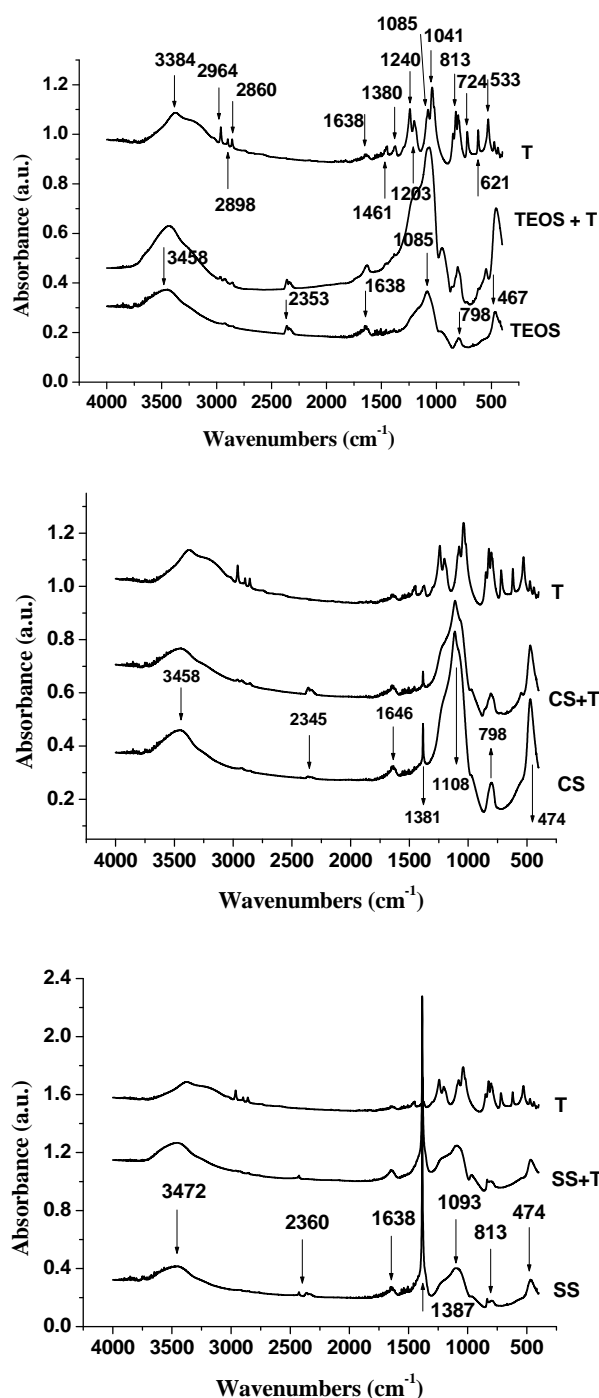
The IR spectra of the prepared samples were compared to the trichlorfon IR spectrum in order to emphasize the fact that, regardless of the SiO<sub>2</sub> precursor, the pesticide was embedded inside the sol-gel matrix. Some of its characteristic vibration bands are absent from the spectra of the composite samples. The assignments of the wave number values from **Figure 1** which refer to the silica matrices proceeding from different precursors are presented in **Table 1**.

The characteristic vibration bands for a SiO<sub>2</sub> gel could be observed, no matter of the used precursor.

Regarding the IR band frequencies of trichlorfon presented in **Figure 1**, they correspond to the following vibrations: OH stretching at 3384 cm<sup>-1</sup>, CH stretching between 2800 and 3000 cm<sup>-1</sup>, OH bending at 1638 cm<sup>-1</sup>, CH deformation vibrations at 1461 and 1380 cm<sup>-1</sup>, PO stretching at 1240 and 1203 cm<sup>-1</sup>, CO stretching at 1085 and 1041 cm<sup>-1</sup>, and C-Cl vibration between 850 and 550 cm<sup>-1</sup>.

From **Figure 1** it can be concluded that the silica matrix, no matter of its provenience, could be an excellent carrier for trichlorfon as embedded organophosphorous pesticide. Subsequent investigations (e.g. release tests) will prove that these matrices are also able to release the incorporated pesticide from their pores.

**Figure 2** presents the comparison between the IR spec-



**Figure 1.** IR spectra of trichlorfon, of the sol-gel pesticide-silica composite samples and of the corresponding silica matrices obtained from different precursors: TEOS, CS, and SS, respectively.

tra of the pesticide-silica composites and those of the corresponding samples in which cyclodextrins have also been included. For the same silica matrix, all the spectra of the samples with cyclodextrins are similar. For this

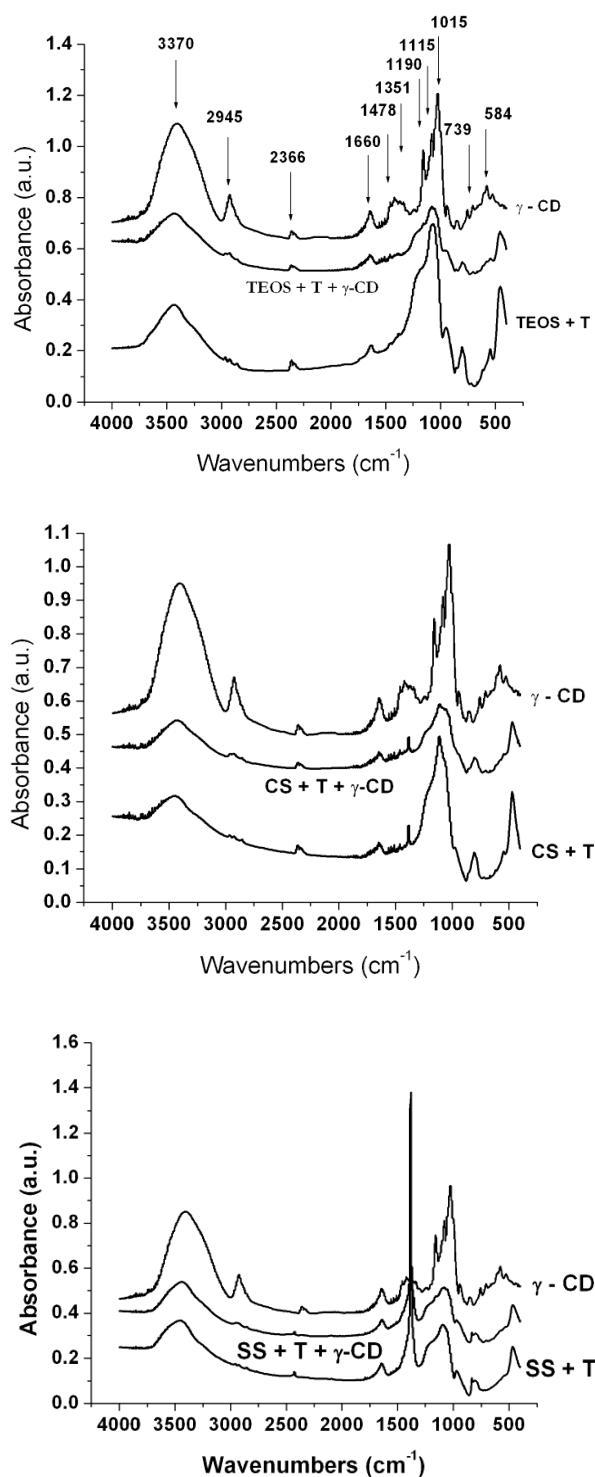
**Table 1.** IR band frequencies and their assignments for the silica gels issued from different precursors (TEOS, CS and SS, respectively).

SiO <sub>2</sub> precursor	Wavenumber [cm <sup>-1</sup> ]	Assignment
TEOS, CS	3458	Structural OH <sup>-</sup>
SS	3472	
SS	2360	CO <sub>2</sub> vib.
TEOS	2353	
CS	2345	
CS	1646	$\delta$ HOH
TEOS, SS	1638	
SS	1387	NO <sub>3</sub> <sup>-</sup> vib.
CS	1381	
CS	1108	$\nu_{as}$ Si-O-Si
SS	1093	
TEOS	1085	
SS	813	$\nu_{sym}$ Si-O-Si
TEOS, CS	798	
CS, SS	474	$\delta$ O-Si-O
TEOS	467	

reason only those containing  $\gamma$ -CD have been presented. The IR spectrum of the  $\gamma$ -CD has also been included.

A very good correspondence between our data referring to the IR spectra of cyclodextrins and the literature ones [43-46] has been obtained. The following IR vibration bands have been registered: OH stretching at 3370 cm<sup>-1</sup>, CH stretching at 2945 cm<sup>-1</sup>, the H bond vibration band at 2366 cm<sup>-1</sup>, OH bending at 1660 cm<sup>-1</sup>, CH deformation at 1478 and 1351 cm<sup>-1</sup>, CO stretching and OH bending at 1190 cm<sup>-1</sup>, CO/CC stretching at 1115 and 1015 cm<sup>-1</sup>, and the pyranose ring vibration at 739 and 584 cm<sup>-1</sup>.

The literature data [47-49] recognize the limits of the IR spectroscopy method regarding the structural investigation of the inclusion compounds of the cyclodextrins. The explanation is simple: the characteristic bands of the cyclodextrins represent an overwhelming part of the complex. Therefore, they are hardly influenced by the complex formation. Only slight alterations of the spectrum of the host could be observed due to the guest molecules, expressed by shifted bands or altered intensities of the IR signals. However, there are some situations in which the cyclodextrins inclusion complexes formation may be proved by the IR spectroscopy analysis [50]. In the present study the identification of the inclusion compounds eventually formed between trichlorfon and cyclo-

**Figure 2.** IR spectra of the sol-gel pesticide-silica composites prepared from different SiO<sub>2</sub> precursors, of the corresponding samples in which  $\gamma$ -CD has been added, and of the  $\gamma$ -CD.

dextrins has become more difficult because of the silica matrix presence.

### 3.2. Thermal Analysis

The use of the thermoanalytical techniques in order to investigate the formation of the cyclodextrins inclusion compounds is well recognized for three decades [41,42, 50,51].

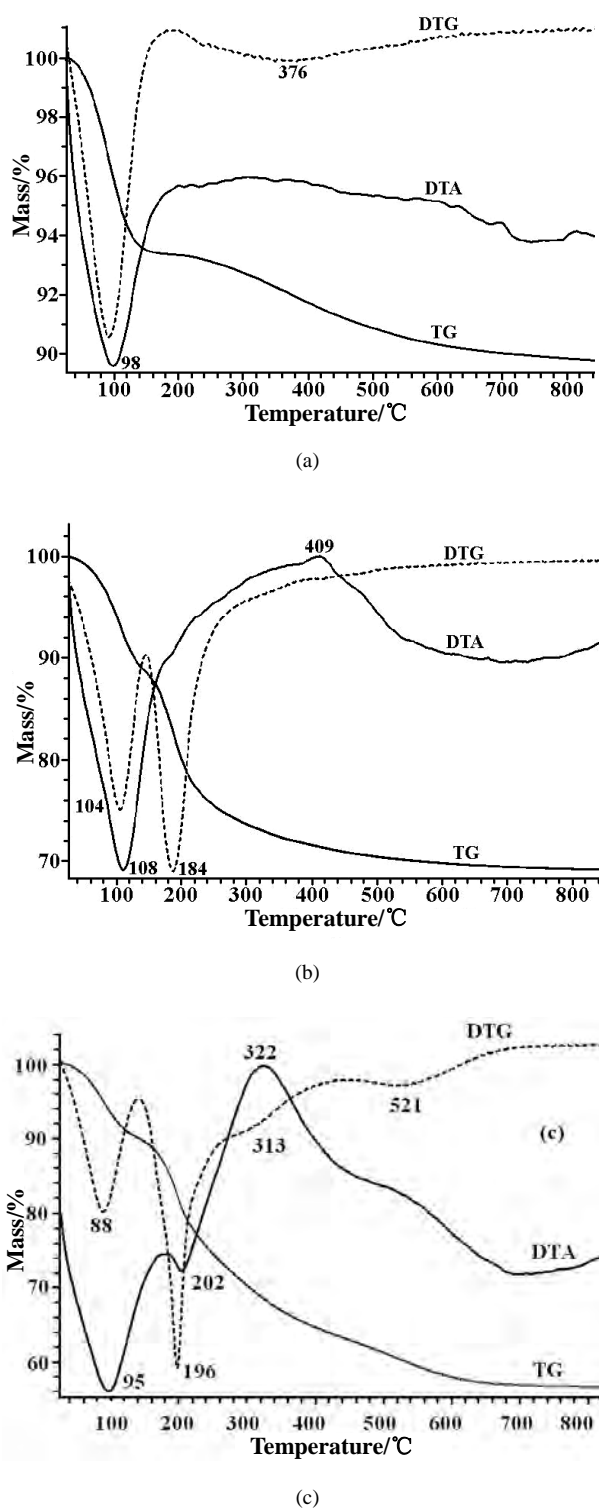
All the prepared samples and the organic compounds included in the sol-gel silica matrices: trichlorfon,  $\alpha$ -,  $\beta$ -, and  $\gamma$ -cyclodextrin, respectively, have been characterized by thermal analysis. **Table 2** and **Figures 3-5** present the obtained results.

In what the three types of cyclodextrins are concerned, the obtained results presented in Table 1 are in agreement with the literature data [41,47,50,52-54].

Because the registered derivatograms of the sol-gel samples obtained with different cyclodextrins ( $\alpha$ -,  $\beta$ -, and  $\gamma$ -CD, respectively) are similar, **Figures 3-5(c)** present only the experimental results obtained using  $\gamma$ -CD.

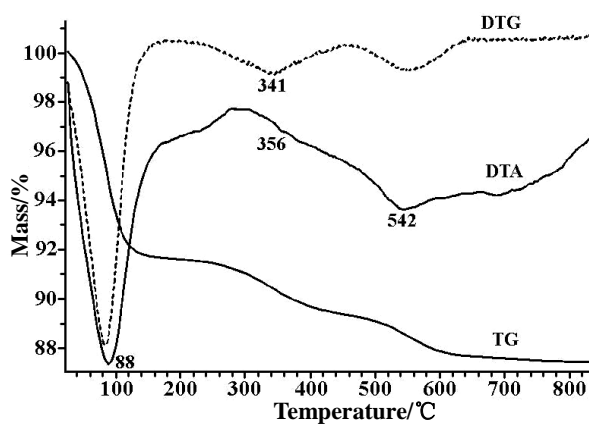
As seen in **Figures 3-5**, the nature of the silica matrix has a significant influence over the thermal behavior of the material. The trichlorfon elimination (which starts in the pure compound at 196°C) is modified in the presence of the matrix: in the case of the organic precursor TEOS, it takes place at 409°C, in the case of the inorganic precursors, the corresponding temperatures are lower: 182°C for the colloidal silica and 245°C, respectively, for the sodium silicate. Regarding the elimination of trichlorfon in the presence of cyclodextrins ( $\alpha$ -,  $\beta$ -, and  $\gamma$ -CD), only in the case of the matrix proceeded from TEOS, the process takes place at temperatures (195-202°C) comparable with those of the pure trichlorfon (196-258°C). In the case of the inorganic  $\text{SiO}_2$  precursors (CS and SS, respectively), the trichlorfon elimination starts at modified temperatures (156-164°C for CS and 155-196°C for SS). The influence of the silica matrix on the thermal behavior of the sol-gel prepared materials can also be seen in the major modification of the temperatures corresponding to the combustion of cyclodextrins. No matter the type ( $\alpha$ -,  $\beta$ -, or  $\gamma$ -CD), they are eliminated by combustion from the samples at much lower temperatures ( $\alpha$ -CD: 305-330°C,  $\beta$ -CD: 295-311°C, and  $\gamma$ -CD: 310-335°C), compared to the temperatures of combustion of the pure cyclodextrins which are around 500°C ( $\alpha$ -CD: 499°C,  $\beta$ -CD: 480°C, and  $\gamma$ -CD: 498°C).

It can be assumed that the shift of the decomposition temperatures is due not only to the silica matrices but also to the inclusion compounds formation. The supposition could be correct for all the sol-gel prepared samples containing both trichlorfon and cyclodextrin, because the melting step corresponding to the trichlorfon could not be observed. In fact, the lack of the melting step of the guest molecule from the derivatograms is considered to be the proof of the inclusion compound formation [41,42,

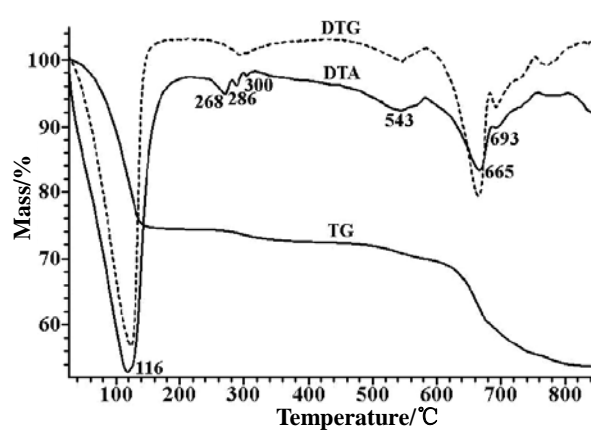


**Figure 3.** Thermal behavior of the silica matrix obtained from TEOS (a) and of the sol-gel samples prepared from TEOS in the presence of T (b) and of both T and  $\gamma$ -CD (c).

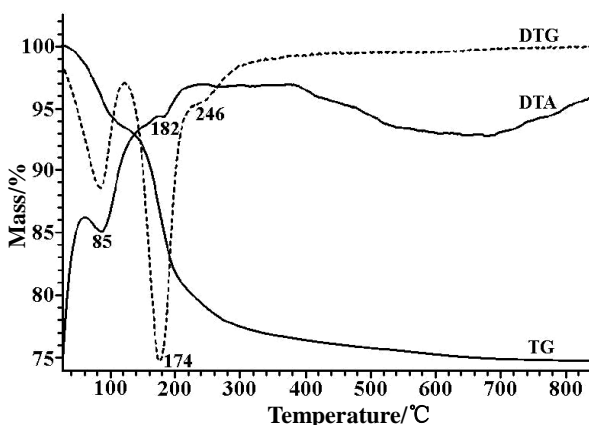
50]. Therefore, it can be concluded that the thermal analysis indicates that the pesticide molecules have in-



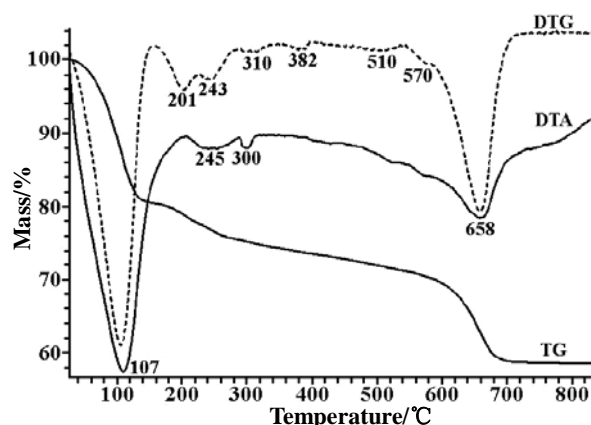
(a)



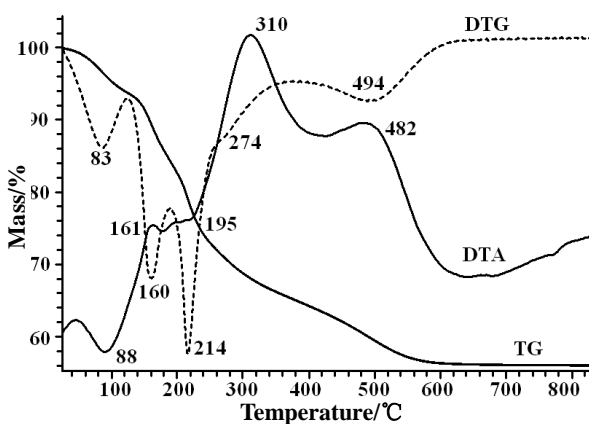
(a)



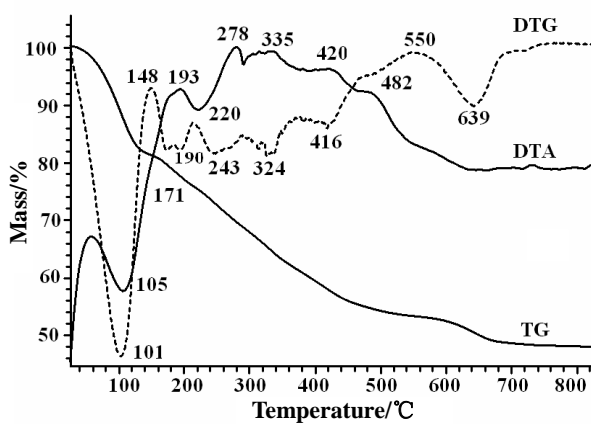
(b)



(b)



(c)



(c)

Figure 4. Thermal behavior of the silica matrix obtained from CS (a) and of the sol-gel samples prepared from CS in the presence of T (b) and of both T and  $\gamma$ -CD (c).

teracted with the cavity of the cyclodextrins host molecules, forming the inclusion complexes. Some doubts still

Figure 5. Thermal behavior of the silica matrix obtained from SS (a) and of the sol-gel samples prepared from SS in the presence of T (b) and of both T and  $\gamma$ -CD (c).

exist, taking into account the fact that in the samples prepared without cyclodextrins, the silica matrix (no

**Table 2. Thermal behavior of the organic compounds used in the sol-gel materials preparation.**

Included organic compound (OC)	Range of temperature [°C]	Temperature of the thermal effect [°C]	Thermal effect	Mass loss [%]		Assignment
				partial	total	
T	20-120	76	endothermic	-		Melting
	120-230	196	endothermic	73.49	94.16	Decomposition
	230-280	258	endothermic	10.27		Decomposition
	280-800			10.40		Decomposition
$\alpha$ -CD	20-140	73, 107	endothermic	9.94		Water elimination
	140-390	274	endothermic	68.24	99.28	Decomposition
	390-800	499	exothermic	21.10		Combustion
$\beta$ -CD	20-130	102	endothermic	13.33		Water elimination
	130-250		endothermic	-	100.18	Melting
	250-390	268	endothermic	64.46		Decomposition
	390-800	480, 532	exothermic	22.39		Combustion
$\gamma$ -CD	20-130	88	endothermic	10.33		Water elimination
	130-390	287	endothermic	70.55	101.10	Decomposition
	390-800	362, 498	exothermic	20.22		Combustion

matter its provenience) seems to be a protection medium for the trichlorfon which doesn't melt when it is included.

In order to clarify this aspect and to evidence the presence of the organic compounds (trichlorfon and cyclodextrins) inside the prepared sol-gel silica matrices, even after their heating up to 800°C, the mass losses corresponding to each of the samples have been calculated from the thermal analysis results. They are presented in **Tables 3-5** for each of the silica precursors used for the preparation of the silica matrix, in order to accomplish a comparative study.

As it can be seen, except for the samples obtained from sodium silicate as precursor of the silica matrix, in all cases the values of "included organic compound" (IOC) were higher than those corresponding to the "total mass loss of the sample" (TML). This fact indicates that the SiO<sub>2</sub> matrices proceeded from TEOS and colloidal silica respectively, are better hosts for the organic materials, compared to that obtained from sodium silicate.

In order to establish both the role of the silica matrix and of the presence of cyclodextrins on the release properties of the materials, the thermal analysis results need supplementary information to elucidate the formation of the inclusion compounds and the properties of the prepared sol-gel samples. Thus, trichlorfon release tests have been accomplished.

### 3.3. Trichlorfon Release Tests

The release of trichlorfon from the sol-gel silica matrices

in the absence and in the presence of cyclodextrins has been assessed by high-performance liquid chromatography (HPLC) measurements.

Relatively recent literature data [7] evidence the fact that although the organophosphorous pesticides (OPP<sub>s</sub>) are among the most widely employed world-wide, only a limited number of representative compounds has been separated by high-performance liquid chromatography (HPLC). Regarding the gas chromatography (GC), the number of publications is even much smaller. The explanation could consist in the fact that the analyses are still based on an empirical selection of the stationary phase, and they depend practically on the experience obtained on a case by case basis. Poor recoveries of compounds such as trichlorfon and dichlorvos (a possible trichlorfon transformation product) have been reported [55]. Despite these informations, in our work trichlorfon release tests have been accomplished, in order to establish that the sol-gel silica matrices could represent new and relatively cheap pesticide carriers with delivery properties. The trichlorfon concentrations have been determined at intervals of 1, 2, 3, 6, 12 and 24 hours, respectively. The experimental results are presented in **Figures 6-8**.

**Figure 6** refers to the sol-gel samples in which the pesticide was embedded in the silica matrices obtained from TEOS and from colloidal silica (CS) in the absence of cyclodextrins. It can be seen that both silica matrices



**Table 3. The mass loss referring to the included organic compounds of the sol-gel prepared materials, using TEOS as SiO<sub>2</sub> precursor.**

Type of included organic compound	Quantity of included organic compound [mg]			Registered mass loss [mg]		Range of temperature [°C]
	T	CD	IOC*	TML**	per stage	
T	13.94	-	13.94	12.38	12.38	140-800
T + $\alpha$ -CD	9.55	12.23	21.78	20.51	11.16	140-280
					4.58	280-420
					4.77	420-800
T + $\beta$ -CD	6.40	8.19	14.59	9.13	4.63	130-280
					2.49	280-420
					2.01	420-800
T + $\gamma$ -CD	7.64	9.78	17.42	14.79	8.05	140-280
					3.53	280-420
					3.21	420-800

\* IOC - The "included organic compound" refers only to the trichlorfon and cyclodextrin introduced in the sample by means of the chemical synthesis. It excludes the organic precursor (TEOS) and the alcohol used in the synthesis as solvent. \*\* TML - The "total mass loss of the sample" is composed from: (a) the mass loss of trichlorfon and cyclodextrin, (b) the organic matter eliminated both from solvent and from the organic silica precursor (TEOS), (c) the mass loss of the water proceeded from the moistures of trichlorfon and cyclodextrins, (d) the mass loss of the water proceeded from the inorganic silica precursors (CS and SS), and (e) the nitrate eliminated from the samples proceeded from SS.

**Table 4. The mass loss referring to the included organic compounds of the sol-gel prepared materials, using CS as SiO<sub>2</sub> precursor.**

Type of included organic compound	Quantity of included organic compound [mg]			Registered mass loss [mg]		Range of temperature [°C]
	T	CD	IOC	TML	per stage	
T	10.92	-	10.92	8.44	5.97	120-220
					2.47	220-800
					5.28	120-190
T + $\alpha$ -CD	10.86	13.89	24.75	20.91	7.04	190-260
					3.62	260-370
					4.97	370-800
T + $\beta$ -CD	6.12	7.83	13.95	11.12	2.80	100-180
					5.98	180-380
					2.34	380-800
T + $\gamma$ -CD	9.87	12.64	22.51	19.53	4.89	120-190
					6.34	190-260
					3.46	260-370
					4.84	370-800

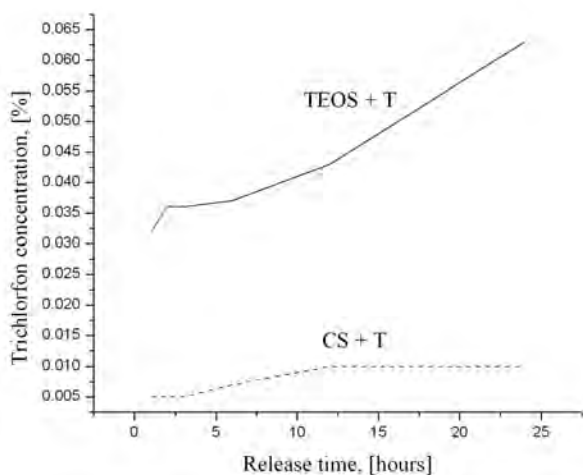
obtained from different SiO<sub>2</sub> precursors have included the pesticide inside their pores, having the property to release it in time. But the release properties in the case of the sample obtained from TEOS precursor were better than in the case of CS.

The corresponding release profile for the sample ob-

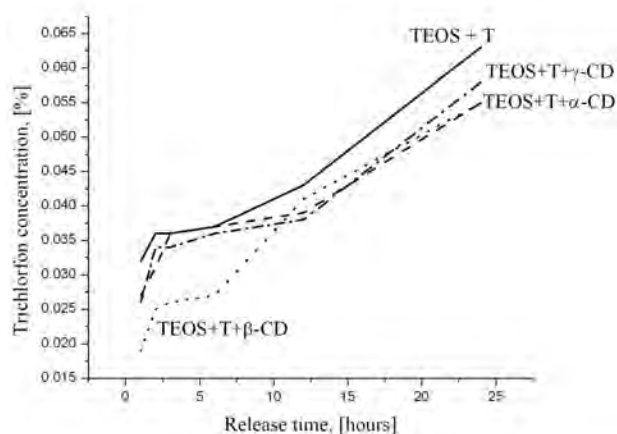
tained from sodium silicate as SiO<sub>2</sub> precursor is absent because the trichlorfon was not detected in this case. Studying the degradation and the half-lives of some organophosphorous pesticides (including trichlorfon and dichlorvos) in water samples, Molina *et al.* [55] show that in many cases transformation products form which

**Table 5.** The mass loss referring to the included organic compounds of the sol-gel prepared materials, using SS as SiO<sub>2</sub> precursor.

Type of included organic compound	Quantity of included organic compound [mg]			Registered mass loss [mg]		Range of temperature [°C]
	T	CD	IOC	TML	per stage	
T	4.41	-	4.41	7.14	2.91	150-520
					4.23	520-800
T + $\alpha$ -CD	4.31	5.51	9.82	11.91	9.73	150-540
					2.18	540-800
T + $\beta$ -CD	2.70	3.46	6.16	7.59	5.90	100-370
					1.69	370-800
T + $\gamma$ -CD	4.45	5.70	10.15	13.09	11.02	150-550
					2.07	550-800

**Figure 6.** The release profiles of trichlorfon for the sol-gel samples proceeding from TEOS and from CS as SiO<sub>2</sub> precursors.

are more stable than the parent compounds. This could explain the lack of the trichlorfon detection in the case of the sol-gel samples proceeding from the sodium silicate precursor. These samples have involved the greatest water quantity for the synthesis. Moreover, the mentioned samples were the only ones which presented the syneresis phenomenon after their gelation. Shchipunov [34] mentions that the volume shrinkage (syneresis) of the synthesized gel after its preparation is of great importance. He considers that the decrease in volume results in the deterioration of the functional characteristics of the embedded organic molecule. This deterioration is attributable to the poor accessibility of substances owing to the diffusion restriction in small pores and could also explain the experimental chromatographic results obtained for the samples proceeding from sodium silicate.

**Figure 7.** The release profiles of trichlorfon for the sol-gel samples proceeding from TEOS in the absence of cyclodextrins and in the presence of  $\alpha$ -,  $\beta$ -, and  $\gamma$ -CD, respectively.

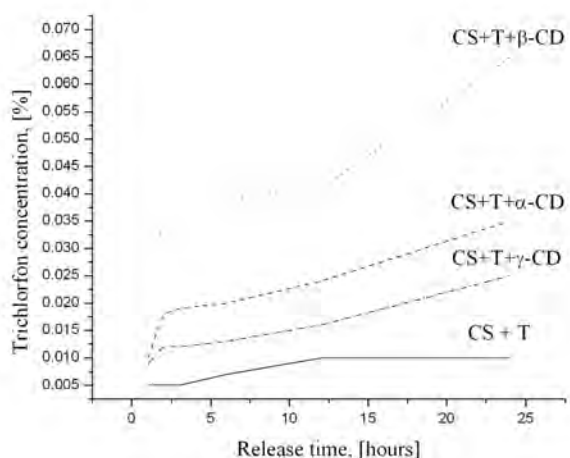
The addition of the cyclodextrins in the sol-gel material formulations modifies their release properties, as it can be seen in **Figures 7 and 8**.

**Figure 7** shows that the addition of cyclodextrins ( $\alpha$ -,  $\beta$ -, and  $\gamma$ -CD) in the sol-gel composite formulation proceeding from TEOS as silica precursor hasn't got a benefic effect, because the released trichlorfon concentration decreases in all cases.

Regarding the materials obtained from colloidal silica, the chromatographic results are presented in **Figure 8**.

**Figure 8** shows another way of how cyclodextrin addition influences the release properties of the prepared materials: no matter the type ( $\alpha$ -,  $\beta$ - or  $\gamma$ -CD), their presence improves the release of the pesticide, increasing the trichlorfon concentration. The best results have been obtained in the case of  $\beta$ -CD addition.

From the presented data it can be concluded that the



**Figure 8.** The release profiles of trichlorfon for the sol-gel samples proceeding from CS in the absence of cyclodextrins and in the presence of  $\alpha$ -,  $\beta$ -, and  $\gamma$ -CD, respectively.

porous sol-gel silica materials generated from different  $\text{SiO}_2$  sources (except the sodium silicate) are able to entrap the organophosphorous pesticide (e.g. trichlorfon) in order to create new controlled release carriers. In all cases (except sample CS + T) the release of trichlorfon in time is slow and continuous during the studied interval of time (1-24 hours). In the absence of cyclodextrins, better results are obtained in the case of the materials obtained from TEOS, compared to those proceeding from colloidal silica as  $\text{SiO}_2$  precursor. The use of cyclodextrins in order to improve the controlled release of the pesticide from the silica matrices showed that only in the case of CS precursor, the addition was successful.

#### 4. Conclusions

Two series of sol-gel composite samples have been prepared, using proper recipes. The first consists in trichlorfon as pesticide, embedded in silica gels generated from different silica sources (TEOS, CS, and SS). The second series is likewise synthesized with the first one. The only difference between them is the presence of cyclodextrins in the reaction mixtures.

The release properties of the prepared materials depend both on the nature of the silica matrix and on the presence or absence of cyclodextrin in the reaction mixture. Thus, best results have been obtained for the samples proceeding from TEOS in the absence of cyclodextrins and for those obtained from CS, in the presence of cyclodextrins.

The delivery properties of the sol-gel silica matrices being confirmed, it can be concluded that they can be considered as a new, ecological type of pesticides carriers.

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# Apoptosis Induced by N-Nitrosamines in Two Cancer Cell Lines

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## ABSTRACT

*In the present study, we investigated the induction of apoptosis by N-nitrosopyrrolidine (NPYR) and N-nitrosodimethylamine (NDMA) in two human cell lines: HL-60 (leukemia) and HepG2 (hepatoma). Apoptotic cells were identified by: 1) chromatin condensation, 2) flow cytometry analysis and 3) poly (ADP-ribose) polymerase cleavage. Both cell lines exhibited morphological changes consistent with apoptotic events following treatment with N-nitrosamines. Flow cytometry analysis showed that both N-nitrosamines induced apoptotic cell death in a concentration and time dependent-manner. NPYR was stronger than NDMA, since it induced a significant apoptotic cell death after 72 h starting from a concentration of 10 mM, whereas NDMA was effective at 27 mM. Furthermore, NPYR and NDMA caused the cleavage of PARP in HL-60 cells whereas no PARP cleavage was detected in HepG2 cells. However, NPYR- and NDMA-induced cell death in HepG2 cells was prevented by specific caspase inhibitors. Caspase-8 mediated main pathway and was responsible for 76% (NPYR) and 64% (NDMA) inhibition of apoptosis. The data demonstrate that NPYR and NDMA induce apoptosis in HL-60 and HepG2 cell lines via caspase-dependent pathway.*

**Keywords:** N-nitrosopyrrolidine, N-nitrosodimethylamine, Apoptosis, Caspases, HL-60 Cells, HepG2 Cells

## 1. Introduction

The N-nitroso compounds (NNC) are recognized as one of the most potent chemical mutagens and carcinogens present in environment and in food [1]. N-nitrosamines are NNC found in foodstuffs, drinking water, rubber products, drug formulations, tobacco and tobacco smoke [2]. Their precursors, nitrites and secondary amines, contained in many common foods, can react under acidic conditions of the stomach and also are produced in vivo by reduction of nitrates by bacteria [3].

The majority of N-nitrosamines tested has been shown to cause cancer at different organs in a variety of animal species and may be causative agents in human cancer [4]. N-nitrosopyrrolidine (NPYR) induces mainly liver tumours in rats [5] and is a weak pulmonary carcinogen in mice [6]. N-Nitrosodimethylamine (NDMA), the simplest and most widely occurring nitrosamine, has been shown to be a potent liver, lung and kidney carcinogen [7]. The metabolic activation of NPYR and NDMA to reactive intermediates by cytochrome P450 is required for the expression of their toxic potential [8]. The key

activation pathway is cytochrome P450-catalyzed hydroxylation of the carbon  $\alpha$  to the nitroso group [9].

Toxic effect of chemicals can lead to passive cell death or necrosis, or result in the active mechanism of apoptosis. Necrotic cell death is an unregulated process resulting from severe damage to the cell and is characterized by ATP depletion, cell swelling, lysis, and the release of intracellular contents resulting in tissue inflammation [10]. Apoptosis, often described as cell suicide [11], is important in carcinogenesis [12], atherogenesis [13] and a number of other diseases [14]. Apoptotic cell death is a complex process characterized by biochemical events and definite morphologic changes [15]. One of the earliest and most consistently observed features is the induction of a series of cytosolic cysteine proteases, known as caspases [16]. Activation of the caspase cascade leads to changes in the plasma-membrane, mitochondria and nucleus [17]. Among the family of ten or more different caspases, already described, caspase-8 and -9 are involved in receptor mediated and intracellular (mitochondrial) pathways of apoptotic cascade, respectively. Different reports indicate that caspase-3, -6 and -7

are the major effector caspases of apoptosis [18].

It is now evident that many environmental chemicals exert their toxicity via apoptotic cell signalling [19]. For instance, it has been demonstrated that numerous food mutagens [20-22] and tobacco specific N-nitrosamine [23] induce apoptosis. Moreover, apoptosis induced by carcinogens seems to have an important role in cancer development. In general, apoptosis of cells exposed to carcinogen compounds allows the removal of cells with extensive DNA damage. However, the removal of these cells may give survival and proliferating signals to the surrounding cells with less DNA damage. This may cause a selection of preneoplastic cells that have become more resistant to carcinogen-induced cell death [24]. NDMA causes the apoptosis of rat neutrophils *in vivo* [25] and their reactive intermediate metabolites cause cell death in P450 2E1-expressing cells by triggering apoptosis [26]. However, to our knowledge, there are not available data about NPYR induced apoptosis.

Thus, the aim of the present study was to investigate the induction of apoptosis by NPYR and NDMA in two human cell lines. The HepG2 hepatoma cell line has been reported to retain some of the drug metabolizing enzyme activities of normal hepatocytes and to increase mRNAs for specific P450 enzymes [27]. Since NPYR and NDMA require metabolic activation catalyzed by the cytochrome P450 for its mutagenicity and carcinogenicity, their actions have been well studied in the liver but there have been few studies in the immune system (blood circulatory system). Moreover, NDMA can influence the activity of the immune system [25]. For this reason, in addition to HepG2 cells, the leukemia cell line HL-60 was tested. This cell line has proven to be a good model for studying the apoptotic process induced by chemicals in lymphoid organs [28] and express relatively high levels of enzymatic isoforms of cytochrome P450 [29].

This study also addresses the role of caspases in N-nitrosamines-induced apoptosis in HL-60 and HepG2 cells.

## 2. Material and Methods

### 2.1. Chemicals

N-nitrosopyrrolidine (NPYR), N-nitrosodimethylamine (NDMA), dimethyl sulfoxide (DMSO), etoposide and acridine orange, were purchased from Sigma-Aldrich, Inc. (St. Louis, MO). The caspase inhibitors, Z-DEVD-FMK (caspase-3 inhibitor), Z-VEID-FMK (caspase-6 inhibitor), Z-IETD-FMK (caspase-8 inhibitor) and Z-LEHD-FMK (caspase-9 inhibitor) were obtained from BD Pharmingen (USA). For western blot analysis, polyclonal poly (ADP-ribose) polymerase (PARP) antibody was purchased from Alexis Biochemicals (Lausen, Switzerland) and

secondary goat anti-rabbit conjugated to peroxidase was obtained from Chemicon (Temecula, CA). All other chemicals and solvents were of the highest grade commercially available.

Standards solutions of NPYR and NDMA (500 mM) were prepared in mili Q water (Millipore, Japan). N-nitrosamines are potent carcinogenic agents, safety precautions were taken for proper handling and disposal of the chemicals.

### 2.2. Cell Lines and Culture Conditions

Human hepatocellular carcinoma cells (HepG2) and human peripheral blood promyelocytic leukemia cells (HL-60) were obtained from the Biology Investigation Center Collection (BIC, Madrid, Spain). HepG2 cells were cultured as monolayer in Dulbecco's Modified Eagle's Medium. HL-60 cells were maintained in RPMI 1640 Medium. The media were supplemented with 10% v/v heat-inactivated fetal calf serum, 50 mg/ml streptomycin, 50 UI/ml penicillin and 1% v/v L-Glutamine. Culture medium and supplements required for the growth of the cell lines were purchased from GIBCO Laboratories (Life Technologies, Inc., Gaithersburg, MD 20884-9980). Controls included a medium control without N-nitrosamines as a negative control. Etoposide has been extensively studied [30] and was used in this study as a positive control (5  $\mu$ M, HL-60 cells; 100  $\mu$ M, HepG2 cells) of apoptosis.

### 2.3. Chromatin Condensation Assay

To examine the effect of N-nitrosamines on nucleus chromatin condensation, HL-60 and HepG2 cells ( $1 \times 10^6$ /ml) were treated with NPYR (10-50 mM) or NDMA (27-135 mM) at different incubation times (24-72 h). After treatments, the cells were stained with acridine orange (5  $\mu$ g/ml) for 10 minutes and observed under a UV-visible fluorescence microscope (Axiostar plus microscope, Zeiss) as described by Gregory *et al.* [31]. Cells exhibiting brightly fluorescent condensed or fragmented nuclei were considered apoptotic. A total of 200 cells were counted in multiple randomly selected fields, and the percentage of apoptotic cells was then calculated.

### 2.4. TdT-dUTP Terminal Nick-End Labeling (TUNEL) Assay

Apoptotic cell death was also measured by the In Situ Cell Death Detection Kit, Fluorescein according to the manufacturer's protocol (Roche, Indianapolis, USA). HL-60 and HepG2 cells were treated with NPYR (10, 30 and 50 mM) or NDMA (27, 68 and 135 mM) for 24, 48 and 72 h. Briefly,  $3 \times 10^6$  cells were washed with PBS and fixed in 2% formaldehyde in PBS (1 ml) for 1 hour



at room temperature. Cells were washed with PBS and incubated with permeabilization solution (0.1% Triton X-100 in 0.1% sodium citrate) for 2 min on ice. Subsequently, the cells were incubated with the TUNEL reaction mixture [50  $\mu$ l of enzyme solution (TdT) and 450  $\mu$ l of label solution (fluorescein-dUTP)] for 1 hour at 37°C in the dark in a humidified atmosphere. During this incubation period, TdT catalyses the addition of fluorescein-dUTP to free 3'-OH groups in single- and double stranded DNA. Omission of TdT from the staining protocol constituted the negative control. After the cells were washed with PBS, the label incorporated into the damaged sites of DNA was detected using a FACS Calibur flow cytometer (Becton and Dickinson) and the CellQuest software. For each experiment  $10^4$  cells were analyzed. To examine the effect of N-nitrosamines on nucleus chromatin condensation, HL-60 and HepG2 cells ( $1 \times 10^6$ /ml) were treated with NPYR (10-50 mM) or NDMA (27-135 mM) at different incubation times (24-72 h). After treatments, the cells were stained with acridine orange (5  $\mu$ g/ml) for 10 minutes and observed under a UV-visible fluorescence microscope (Axiostar plus microscope, Zeiss) as described by Gregory *et al.* [31]. Cells exhibiting brightly fluorescent condensed or fragmented nuclei were considered apoptotic. A total of 200 cells were counted in multiple randomly selected fields, and the percentage of apoptotic cells was then calculated.

## 2.5. Western Blot

After incubation of cells with NPYR (10, 30 and 50 mM) or NDMA (27, 68 and 135 mM) for 24-72 h (HepG2), protein extracts were obtained with Nucleus Buster Protein Extraction Kit (Novagen, Darmstadt, Germany). Equal amounts of protein cell extracts (30  $\mu$ g) measured by the Non Interfering Protein Assay Kit (Calbiochem) were used for western blot analyses. Samples were resuspended in a buffer containing 63 mM Tris-HCl, pH 6.8, 10% glycerol, 1% 2-mercaptoethanol, 2% sodium dodecyl sulfate (SDS) and 0.025% bromophenol blue and boiled for 15 min. Proteins were resolved on a 10% sodium dodecyl sulfate-polyacrylamide gel and electroblotted onto an immune-blot PVDF membrane (Bio-Rad Laboratories) at 119 V for 1 hour and 15 min in Tris glycine buffer (25 mM Tris, 192 mM glycine, 20% methanol, pH 8.3). Equal protein loading and the integrity of transfer were confirmed by Blot-Fast-Stain (Chemicon, Temecula, CA). Subsequently, the membranes were blocked overnight in milk block buffer (PBS, 0.2% Tween, 10% non fat dry milk) and then incubated with polyclonal poly (ADP-ribose) polymerase (PARP) antibody diluted 1:1000 in milk block buffer on a plate shaker for 1 h at room temperature. The membranes were then washed three times (10 min each) in milk block

buffer, and goat anti-rabbit peroxidase conjugated diluted 1:3000 in milk block buffer was applied to the blots for 1 h at room temperature with shaking. Then, the blots were washed as described above and one more time with PBS-Tween. Blots were developed using the super signal substrate (Pierce, Rockford, IL) and chemiluminescence was directly detected using Bio-Rad Fluor S instrument and analysed using the Bio-Rad quantity one software package.

## 2.6. Caspase Activity

To address the significance of caspases activation in N-nitrosamines-induced apoptosis in HepG2 cells, we used permeable, specific and potent caspase inhibitors, Z-DEVD-FMK (caspase-3 inhibitor), Z-VEID-FMK (caspase-6 inhibitor), Z-IETD-FMK (caspase-8 inhibitor) and Z-LEHD-FMK (caspase-9 inhibitor). HepG2 cells were treated with 50 mM NPYR (48 h) or 68 mM NDMA (72 h) in the presence or absence of 100  $\mu$ M of caspase inhibitors. After the incubation, the percentage of apoptotic cells was determined by TUNEL assay and flow cytometry.

## 2.7. Statistical Analyses

The Student's t-test was used for statistical comparison and differences were considered significant at  $p \leq 0.01$ . Descriptive and graphical methods were used to characterize the data. All tests were performed with the software package Statgraphics Plus 5.0.

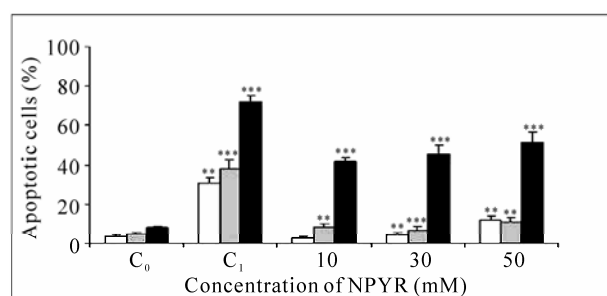
# 3. Results

## 3.1. Analysis of Morphological Changes Induced by NPYR and NDMA

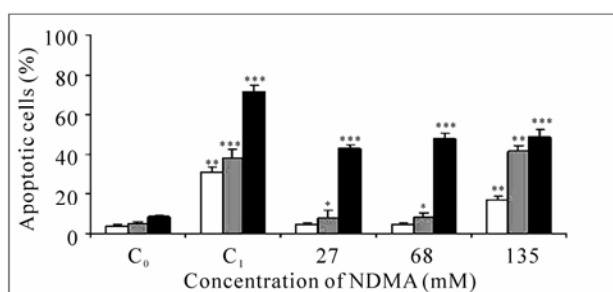
Initial studies were performed to investigate whether NPYR and NDMA-induced apoptosis in human cancer cells. Thus, cells were treated with 10-50 mM NPYR or 27-135 mM NDMA for different time periods (24-72 h), and nuclear morphology was observed by fluorescence microscopy using acridine orange. 24 hours treatment of HL-60 cells at the highest doses of NPYR (50 mM) and NDMA (135 mM) induced 58% and 39% of apoptosis, respectively. In HepG2 cells, 24 h of treatment with NPYR (50 mM) and NDMA (135 mM), induced above 62-46% of apoptosis, respectively.

## 3.2. TUNEL Assay

The TUNEL assay is a sensitive test to detect the DNA strand breaks that are a hallmark of the late stages of apoptosis [32]. TUNEL analysis showed that NPYR and NDMA-induced apoptosis in HL-60 (**Figure 1**) and HepG2 cells (**Figure 2**), in a concentration and time dependent-manner. An increase in the number of apoptotic (41-



(a)



(b)

**Figure 1.** Flow cytometric analysis using TUNEL assay of HL-60 cells treated with different concentrations of NPYR (a) and NDMA (b) for 24 h (□), 48 h (▒) and 72 h (■). C<sub>0</sub>, untreated cells; C<sub>1</sub>, cells treated with etoposide (5 μM). Asterisks indicate significant difference from control \*\*\*  $p \leq 0.001$ , \*\*  $p \leq 0.01$  and \*  $p \leq 0.05$ .

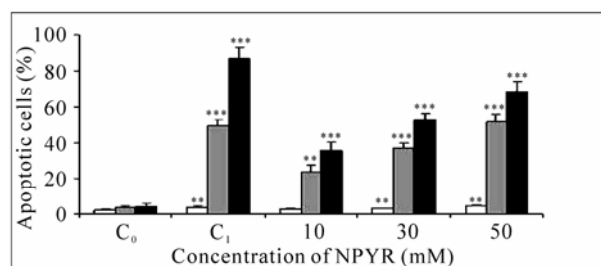
51%) HL-60 cells was apparent after 72 h incubation with 10-50 mM NPYR (**Figure 1(a)**). The short-time treatments (24 and 48 h) with NDMA (27 and 68 mM), did not induce a significant percentage of apoptotic cells (4%), whereas 72 h induced about 43-48%, respectively (**Figure 1(b)**). After treatment with 135 mM of NDMA, an increase in the percentage of apoptotic cells was detected from 48 to 72 h of incubation (41-49%, respectively).

The presence of apoptotic cells was noted in HepG2 cells treated with NPYR for 48 h (24-52%) (**Figure 2(a)**). When the cells were treated for 72 h, the highest number of apoptosis was induced with 50 mM of NPYR (68%).

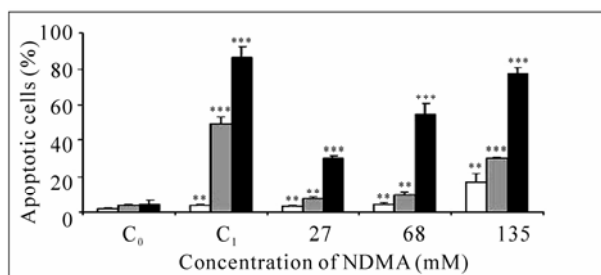
As shown in **Figure 2(b)**, only a moderate number of TUNEL positive cells were observed after treatment with NDMA for 24 and 48 h. However, at 72 h, a remarkable percentage of apoptotic cells occurred at all concentrations of NDMA. More than 70% of HepG2 cells were TUNEL positive after treatment with 135 mM of NDMA.

### 3.3. Western Blot

In view of the ability of NPYR and NDMA to induce apoptosis by the TUNEL assay, it was considered of in-



(a)

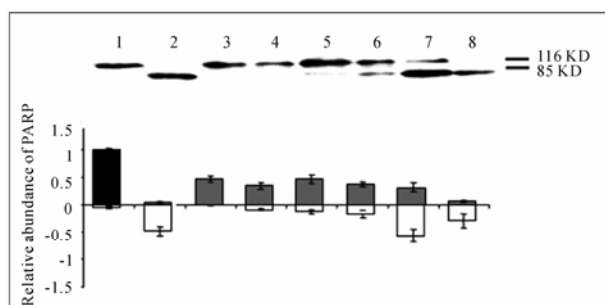


(b)

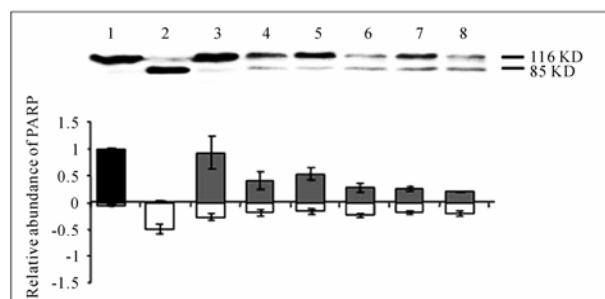
**Figure 2.** Flow cytometric analysis using TUNEL assay of HepG2 cells treated with different concentrations of NPYR (a) and NDMA (b) for 24 h (□), 48 h (▒) and 72 h (■). C<sub>0</sub>, untreated cells; C<sub>1</sub>, cells treated with etoposide (100 μM). Asterisks indicate significant difference from control \*\*\*  $p \leq 0.001$  and \*\*  $p \leq 0.01$ .

terest to examine the role of caspases. PARP is a preferential substrate for caspase-3 and is cleaved by this protein into 85 and 24 kDa fragments during the apoptotic mode of cell death [33]. Thus, protein extracts from HL-60 and HepG2 cells untreated and treated with NPYR, NDMA and etoposide, were electroblotted and probed against a PARP polyclonal antibody that recognizes the 116-kDa intact PARP as well as an 85-kDa cleaved product. Quantification of PARP cleavage was determined by densitometry of the intensity of full-length protein signal visualized by polyclonal anti-PARP antibody.

As shown in **Figure 3**, untreated HL-60 cells showed only intact PARP at 116 kDa (**Figures 3(a)** and **3(b)**, lane 1). In contrast, all the PARP present in the 5 μM etoposide-treated cells had been cleaved into the 85 kDa fragment (**Figures 3(a)** and **3(b)**, lane 2). No PARP cleavage was detectable after incubation of cells with 10 mM NPYR (**Figure 3(a)**, lanes 3 and 4). Treatment of HL-60 cells with 30 mM NPYR induced the cleavage of PARP from 116 to 85 kDa at 48 h (**Figure 3(a)**, lanes 5 and 6). Some uncleaved PARP remained in 50 mM NPYR treated HL-60 cells at 24 h, whereas a PARP cleavage product of 85 kDa was prominent at 48 h (**Figure 3(a)**, lanes 7 and 8). As shown in **Figure 3(b)**,



(a)



(b)

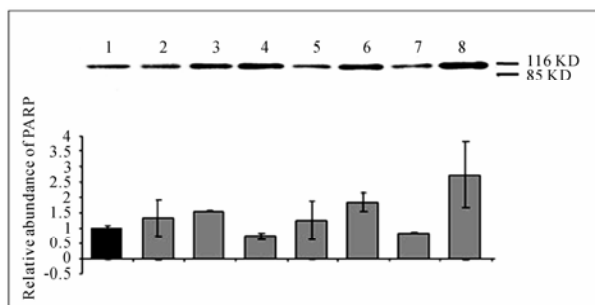
**Figure 3.** Western blot of PARP cleavage in HL-60 cells treated with NPYR (a) and NDMA (b). Lane 1 represents untreated cells and lane 2 represents cells treated with etoposide. Lane 3 cells treated with (a) 10 mM or with (b) 27 mM for 24 h and lane 4 for 48 h. Lane 5 cells treated with (a) 30 mM or with (b) 68 mM for 24 h and lane 6 for 48 h. Lane 7 cells treated with (a) 50 mM or with (b) 135 mM for 24 h and lane 8 for 48 h.

treatment of the cells with NDMA caused the proteolytic cleavage of PARP with accumulation of an 85 KDa fragment and the concomitant disappearance of the original 116 KDa PARP at all test doses.

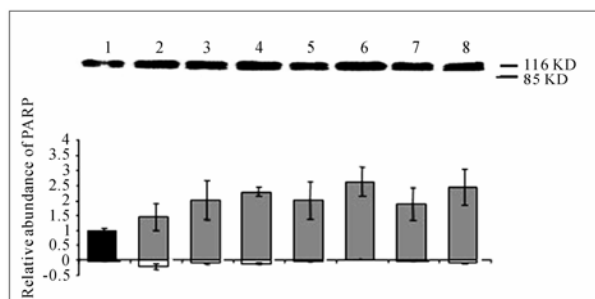
**Figure 4** shows the results obtained in HepG2 cells treated with NPYR and NDMA. In HepG2 cells untreated and treated with etoposide (100  $\mu$ M for 24 and 72 h), western blot revealed only a single band at 116 KDa representing full-length enzyme (**Figures 4(a) and 4(b)**, lanes 1 and 2). Similarly, PARP cleavage could not be detected after the treatment of HepG2 cells with different doses of NPYR or NDMA for 24 to 72 h (**Figures 4(a) and 4(b)**, lanes 3 to 8).

### 3.4 Effects of NPYR and NDMA on the Caspase Pathway in HepG2 Cells

Since the key effector molecules of the apoptotic process belong to the caspase family, we evaluated the ability of NPYR (50 mM, 48 h) and NDMA (68 mM, 72 h) to induce apoptosis in HepG2 cells in the presence or absence of different caspase inhibitors (100  $\mu$ M).



(a)



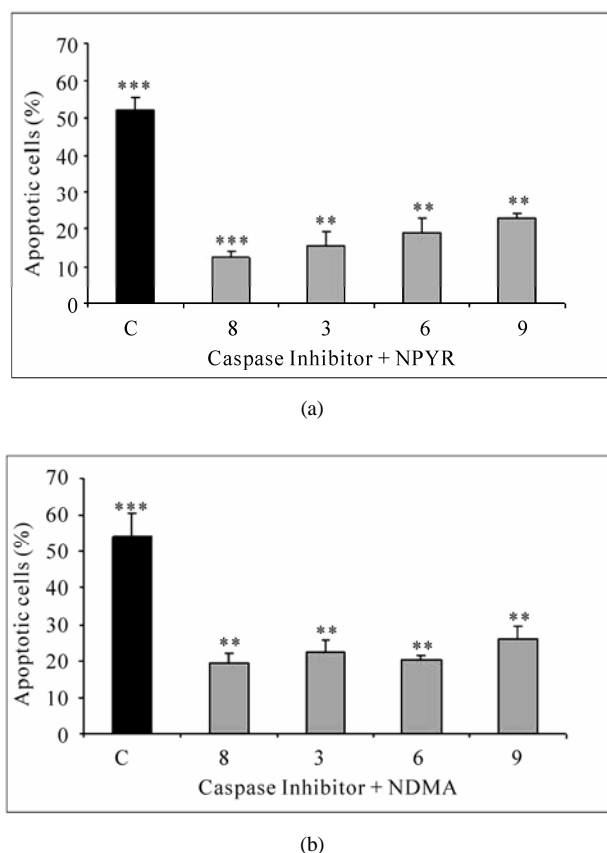
(b)

**Figure 4.** Western blot of PARP cleavage in HepG2 cells treated with NPYR (a) and NDMA (b). Lane 1 represents untreated cells and lane 2 represents cells treated with etoposide. Lane 3 cells treated with (a) 10 mM or with (b) 27 mM for 24 h and lane 4 for 72 h. Lane 5 cells treated with (a) 30 mM or with (b) 68 mM for 24 h and lane 6 for 72 h. Lane 7 represents cells treated with (a) 50 mM or with (b) 135 mM for 24 h and lane 8 for 72 h.

As shown in **Figure 5**, the addition of Z-IETD-FMK (caspase-8 inhibitor) significantly diminished NPYR- and NDMA-induced apoptosis in a 76-64%, respectively. The Z-DEVD-FMK (caspase-3 inhibitor) reduced the apoptotic effect of NPYR and NDMA in 79-58%, respectively, and Z-VEID-FMK (caspase-6 inhibitor) inhibited a 63% both N-nitrosamines. The blockage of apoptosis by Z-LEHD-FMK (caspase-9 inhibitor) caused an inhibition of NPYR- and NDMA-induced apoptosis of 56 and 52%, respectively.

## 4. Discussion

Apoptosis induced by carcinogens seems to have an important role in cancer development [34]. Accordingly, the mechanism and cell signalling pathways involved in food carcinogens-induced cell death or cell survival and proliferation have recently received much interest [26,35]. In 1978 the International Agency for Research on Cancer (IARC) classified NPYR and NDMA as possibly and probably carcinogenics to humans, respectively [36]. It is widely accepted that N-Nitrosamines require metabolic



**Figure 5. Effect of specific caspase inhibitor on apoptosis induced by (a) 50 mM NPYR (48 h) or (b) 68 mM NDMA (72 h) in HepG2 cells, using TUNEL assay and flow cytometry. C (■), HepG2 cells treated with N-nitrosamines and without caspase inhibitor. (□) HepG2 cells treated with N-nitrosamines and specific caspase-3, -6, -8 or -9 inhibitor. Asterisks indicate significant difference from control \*\*\*  $p \leq 0.001$  and \*\*  $p \leq 0.01$ .**

activation by cytochrome P-450 to become carcinogenic. The activated N-Nitrosamine attacks and covalently binds to DNA, forming detectable DNA adducts. Wang *et al.* [37] showed that there was a significant positive association between NPYR exposures and having detectable several adducts in hepatic DNA of rats. Moreover, Cheng *et al.* [38] have just demonstrated that NDMA damage calf thymus DNA through reactive metabolites. DNA lesions are converted into double strand breaks that act as a trigger for the up-regulation of p53, which induces apoptosis by the cell death receptor pathway [39].

In the present study, a variety of methods have been employed to detect and quantify apoptosis, since many studies suggest that the utilisation of two or more different techniques may be convenient to avoid determination errors [40,41]. Our results clearly demonstrated that NPYR and NDMA-induced apoptosis in a concentration

and time dependent-manner as judged by the TUNEL assay (Figures 1 and 2). Moreover, we observed that HepG2 cells were more sensitive to the treatments than HL-60 cells. At 72 h, 50 mM NPYR induced 51% of apoptosis in HL-60 and 68% in HepG2 cells, whereas 135 mM NDMA caused 49% and 78% of apoptosis in HL-60 and HepG2 cells, respectively. It has been reported that cell lines differ substantially in their sensitivity towards various classes of apoptotic chemicals [42]. Thus, Duc and Leong-Morgenthaller [43] found that the heterocyclic amine 2-amino-1-methyl-6-phenylimidazo [4, 5-b]pyridine (PhIP) induced different apoptotic response in two human lymphoblastoid cell lines (TK6 and MT1). A five to six fold increase and less than a two fold increase in the fraction of apoptotic cells were observed in TK6 and MT1, respectively. In addition, in our previous studies [44,45] HepG2 cells were more resistant to the apoptosis induction by N-nitrosodibutylamine (NDBA) and N-nitrosopiperidine (NPIP) than HL-60 cell line. N-nitrosamines are metabolised by enzymes of the mixed-function cytochrome P-450-dependent monooxidase system. Cyclic N-nitrosamines such as NPYR and NPIP are primarily activated by CYP2A6. On the other hand, short chain N-nitrosamines such as NDMA is activated by CYP2E1 whereas CYP1A1 is involved in the metabolism of the longer chain N-nitrosamines such as NDBA [46]. Thus, a possible explanation of the variation in the percentage of apoptotic cells induced by N-nitrosamines could be attributed to the differences in the levels of enzymatic activities in both cell lines.

Both N-nitrosamines induce apoptosis in HL-60 and HepG2 cells, even though NPYR was most effective than NDMA at lower concentration. Numerous studies have reported that genotoxicity of NDMA in human hepatoma cell lines was observed only at high concentrations [47, 48]. Furthermore, we have demonstrated that NPYR exerted greater oxidative DNA damage in HepG2 cells than NDMA by using the Comet assay [49]. The lowest concentration of NDMA required to cause a significant increase in DNA damage was approximately 5-fold higher than that of the corresponding NPYR. In comparison with our previous studies [44,45] NDBA was the most effective N-nitrosamine to induce apoptosis in both cell lines by the TUNEL assay. Thus, after 24 h incubation with NDBA at 3.5 mM, the percentage of apoptotic HepG2 cells reached 95%, whereas it was necessary to use doses of 45 mM NPIP (86%), 50 mM NPYR (68%) and 135 mM NDMA (77%) and longer incubations times (72 h) to obtain a high percentage of apoptotic HepG2 cells. Similar findings have been obtained in the leukemia HL-60 cell line. At 72 h, 2.5 mM NDBA induced 69% of apoptotic HL-60 cells, whereas it was necessary doses of 20 mM NPIP (75%), 50 mM NPYR (51%) and

135 mM NDMA (49%) to obtain a high percentage of apoptotic cells. The fact that the percentage of apoptotic cells varied with the type of N-nitrosamine suggests that the apoptotic effect depended on the chemical structure of N-nitrosamine.

Activated caspase-3 cleaves PARP, a 116 kDa enzyme, generating a fragment of 85 kDa [50]. Our results showed that NPYR and NDMA caused PARP cleavage in HL-60 cells, in a concentration and time dependent manner (**Figure 3**). In contrast, no PARP cleavage (reflecting caspase-3 activation) was detected in HepG2 cells (**Figure 4**). These results are in agreement with Di-bartolomeis and Moné [51] who assumed that the PARP cleavage was based on the disappearance of the 116 kDa fragment in Jurkat cells treated with with 500  $\mu$ M etoposide.

To determine whether the caspases were involved in NPYR and NDMA-induced apoptosis in HepG2 cells, we also analysed the effects of the specific inhibitors of caspase activity. The two major apoptotic pathways described in eukaryotic cells are extrinsic (triggered by death receptors) and intrinsic mediated by mitochondrial events [52]. The apical proteases in the extrinsic and intrinsic pathways are caspase-8 and caspase-9, respectively. Activated caspase-8 and -9 further initiates the activation of caspase cascade leading to biochemical and morphological changes associated with apoptosis [53]. Caspase-3 and -6 are well-known downstream effector caspases which can be proteolytically activated by caspase-8 or -9 via different signalling pathways [54].

Our results confirmed that NPYR and NDMA-induced cell death in HepG2 cells was due to caspase-dependent apoptosis (**Figure 5**). Caspase-8 seems to be the central caspase in the NPYR and NDMA-induced apoptosis because blocking of its activity had the highest percentage of reduction of apoptosis (76-64%, respectively). Inhibition of caspase-3 and -6 activities partially inhibited the NPYR and NDMA-induced apoptosis which suggested that caspase-8 was upstream of caspase-3 and -6. However, may be other mechanism for caspase-3 and -6 activations, apart from the cascade mediated through caspase-8 activation [55]. Caspase-9 activity was slightly reduced by the inhibitor, suggesting the involvement of intrinsic pathway, but might not be the major way to induce apoptosis in HepG2 cells. It is now recognized that some chemicals induce apoptosis via the mitochondria-dependent pathway in which caspase-9 is initially activated [56] and also activate caspase-8 in the absence of the death receptor signalling [57]. Our results are consistent with the report in which heterocyclic amine, 3-Amino-1,4-dimethyl-5H-pyrido-[4,3-b]indole (Trp-P-1)-induced apoptosis, mainly operates the caspase-8-depen-

dent pathway, and there is also a caspase-9-dependent side pathway [21]. Moreover, we found that both the intrinsic and extrinsic pathways were similarly involved in the NPIP and NDBA-induced apoptosis in HepG2 cells [45].

Taken together, the results reported in this work demonstrate that NPYR and NDMA induce apoptosis in HepG2 and HL-60 cell lines via caspase dependent pathway. Further studies are needed to determine the molecular mechanism of NPYR and NDMA induce apoptosis.

## 5. Acknowledgements

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# An Analysis of the Benefit on Green Risk in Construction Projects

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## ABSTRACT

The Construction project green risks (CPGRs) refer to those threats to environment, energy sources and material resources during the entire life-cycle of a construction project. The emergent green risks in exploiting these resources are of varying concern to all. In this paper, evolutionary game is introduced to make about impacts of strategy choices from interactions among the choices developers, and between the choices developers and EPDs on project green risk. The results show that CPGRs will occur if either developers find that not managing CPGRs has a better payoff than opting for CPGR management, or if monitors impose only mild fines even when they find CPGRs within construction projects of developer's firms. The study also shows that there is a prohibitively expensive cost incurred by EPDs in monitoring CPGRs. Finally, some strategies are given for EPDs to help them make policies to regulate the strategies of developers.

**Keywords:** Construction Project Management, Green Risk, Evolutionary Game, Strategy Developer

## 1. Introduction

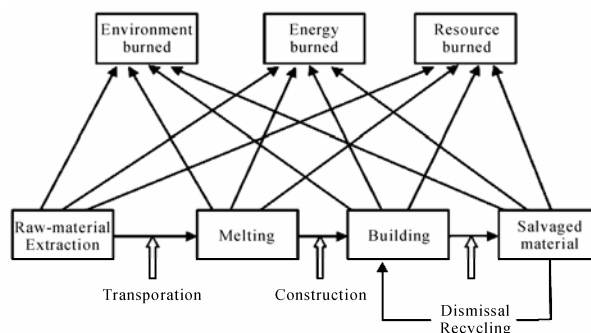
### 1.1. Construction Project Green Risk

Construction is a process of building in which materials are transformed into products, e.g. buildings, airports and highways, which inevitably leads to some form of environmental pollution, energy consumption and resource depletion. Up to date Chinese government has called for “energy-saving and pollution-reducing” initiatives. Such a policy is helpful to prod construction project green risk developer (CPGR-developers) to have concern for the environment and to manage green risks. However, the validity of such policies has yet to not be seen.

Green risks (GRs) are those threats to human beings and/or to what they value, from hazards, either natural or human-driven, associated with global change [1]. CPGRs refer to those threats to environment, energy and resources which occur during the entire life cycle of construction project, and the process is depicted as **Figure 1**.

### 1.2. Brief View of CPGRs Researches

A necessary question to pose is how to raise awareness toward CPGRs and with collaboration of the construction sector to promote solutions that better conservation policies. There two viewpoints about CPGRs, and some researchers hold views that lowering CPGRs can resort to



**Figure 1. The impacts on environment, resource and energy.**

reducing building materials consumption [3-11]; but others thought although using less material can avert CPGRs yet this response could sacrifice some building functions, and so they suggested an alternative approach based on boosting the cost of introducing CPGRs, which advocating government or EPDs taxing or penalising much more than previous if an enterprise creates or elevates CPGRs, such as raising the resource production price, reducing the overall energy amount to be used by an enterprise, penalising pollution violators, to block the projects from proceeding. Further they have analysed CPGRs based on game theory [12-14], and believed that heavy fines will encourage construction managers to deal with green risk,

compel them to make a decision either to comply with environmental policy decreed by government to better management of CPGRs, or to face penalties from EPDs and incur financial cost or some other such punishment.

Unfortunately traditional game theory is usually limited to exploring rational agents' interactions. However, the rational assumption is not valid for CPGR-developers in that these developers will find it difficult to know competitors' strategies and hence their own decisions will not be treated rationally due to incomplete knowledge. Thus bounded rationality, as it is called, is the norm, and over time CPGR-developers will learn and develop their own strategies, or imitate other players' strategies. Indeed, they may change them constantly and hence develop an evolutionary stable strategy (ESS). In this article, evolutionary game is introduced to analyse the choice behaviours, by considering decision-makings of CPGR developers and their interactions among each other and with EPDs, in order to find motives and reasons to behaviours towards CPGRs.

## 2. The Evolutionary Game Based Model of CPGR Developers

Confronted with CPGRs, developers (those firms who by their activity in the construction area produce the CPGRs) have two choices. One choice, which here is called *manag*, is using new technology to reduce green risk. The technology may take the form of utilizing low-energy consumption machines, providing low-energy steel, or increasing the use of recycled metals. The other choice is *non-manag*, i.e., in plain speak, shirking responsibility for green risk and simply doing nothing. Now we assume that there exists a construction project for which there are two vying construction firms that have presented project tenders. Within the game theory context, we introduce outcomes determined by the attitude of the developers to CPGRs. If a developer elects to deal with them the gain is  $A$ ; if the other does nothing, the developer gets  $B$ , otherwise  $C$ . Of course, the two developers will both get  $D$  if they show no concern toward CPGRs. The payoff matrix that game theory ascribes to the two players of this game is as follows **Table 1**.

For the two players, the problem is which strategy should be employed; the decision depends on the action

of the other; such a situation illustrates a game of imperfect information. As a consequence, we rank the pay-offs as  $A \neq B \neq C \neq D$ . If two players are rational economic agents, the result of the game depends on the relative values assigned to  $A$ ,  $B$ ,  $C$  and  $D$ . However, the developers are in fact bounded rational agents, so after playing the game, the values  $A$ ,  $B$ ,  $C$  and  $D$  must have some relevance to reality, so that evolutionary game theory is applicable.

In this game, the two developers choose between two strategies with a certain payoff. However, how will a population of developers that repeatedly play this game evolve? In what follows, it will be assumed that the pay-offs will be the same for every developer. We cannot answer the above question without introducing some assumptions concerning the nature of the population. Let us begin by assuming the number of developers is large, so that we can represent the state of the population by keeping track of what proportion of developers follow the strategy *management* and those following *non-management*. We further assume that the proportions following a particular strategy at the next generation of play is proportional to that of the current generation. Thus the strategies themselves are now playing each other. This then provides us with differential equations and hence continuous dynamics known as replicator dynamics for an evolutionary gaming theory. Finally we assume that strategies are uncorrelated, i.e. that the probability with which every strategy meets every other strategy depends only on the relative frequencies within the population. Thus the games between developers are randomly played.

We denote the frequency of population in management strategy in the CPGR decision game as  $x$ , which will vary with time  $t$ , and consequently the population ratio of non-management strategies with  $1 - x$ , and then we have:

$$\begin{cases} u_t = xA + (1-x)B \\ u_f = xC + (1-x)D \\ \bar{u} = xu_t + (1-x)u_f \end{cases} \quad (1)$$

where  $u_t$  is the expected gain for the management strategy,  $u_f$  is the expected gain for the non-management strategy,  $\bar{u}$  is the average gain for all construction firms for the project at that generation.

Based on  $2 \times 2$  symmetric game model, we can get dynamic Equation (2), then obtain Equation (3) if substituting (1) into, and further induce Equation (4) and three possible stationary states are eventually obtained if setting  $F(x) = dx/dt = 0$ . According to evolutionary stable strategy (ESS) of the differential equation, we obtain the optimal solution  $x_i^*$  is ESS, if  $F'(x_i^*) = 0$ . The equations listed as follows:

**Table 1. Payoff matrix of CPGR-developers.**

		CPGR-Developer II	
		Manag.	Non-manag.
CPGR-Developer I	Manag.	$A, A$	$B, C$
	Non-manag.	$C, B$	$D, D$

$$dx/dt = x(u_t - \bar{u}) = x(1-x)(u_t - u_f) \quad (2)$$

$$F(x) = dx/dt = x(1-x)[x(A-C) + (1-x)(B-D)] \quad (3)$$

$$\begin{cases} x_1^* = 0, x_2^* = 1, \\ x_3^* = \frac{D-B}{A-B-C+D} \text{ if } 0 \leq \frac{D-B}{A-B-C+D} \leq 1 \end{cases} \quad (4)$$

In the model there are four aspects needed to be focused as follows:

The circumstance  $A = C$  and  $B = D$  implies one player, who is playing management strategy, will receive more gain, irrespective of whether the other players are executing management strategy or not. This situation will occur if government can strictly enforce laws concerning CPGR-developers. If this is the case, the risks can be determined and developers will be punished. The loss resulting from punishment is higher than the cost of managing the risks. Thus,  $F'(x_1^*) = 0$ ,  $F'(x_2^*) < 0$ ,  $x_3^*$  is not ESS,  $x_2^* = 1$  is the only ESS. Therefore, we can describe the game result as one in which a bounded rational developer, after playing repeatedly, will change to management strategy. This selection will encourage management strategy as the cleverer scheme for developers.

It is possible that one player who opts for non-management strategy will gain more whether or not it is played by the other player. This situation requires  $A < C$  and  $B < D$ . Normally, this state arises when the EPDs are completely in breach of their duty and hence the ignored CPGR-developers will find that there would be more gain if they played non-management strategy rather than management strategy. In this case  $x_1^* = 0$  is the only ESS because  $F'(x_1^*) < 0$  and  $F'(x_2^*) = 0$ . Playing the game repeatedly, bounded rational developers will think it is foolish to elect management strategy.

If one player chooses management strategy in this CPGR decision game, the other player then finds management strategy is a smart choice for himself, as in this situation if  $A = C$  and  $B < D$  is satisfied then one player receives less gain from management strategy than from the non-management strategy when the other player does nothing about CPGRs. These cases will occur repeatedly if the non-management action earns more benefit than the loss incurred in the punishment from EPDs. Of course,  $F'(x_1^*) < 0$ ,  $F'(x_2^*) < 0$ ,  $F'(x_3^*) = 0$ ,  $x_1^* = 0$  and  $x_2^* = 1$  are ESSes. Thus the game result will be decided on the original population level of  $x$ . When  $x \in (0, x_3^*)$ , after repeated play, the developer will give up management strategy. However, when  $x \in (x_3^*, 1)$ , the contrary situation will be taken up. Especially, if  $D = B$ ,  $A \neq C$ , thus  $x_3^* = 0$ , all the developers will choose non-management plan and the management strategy is used when  $D \neq B$  and  $A = C$  because of  $x_3^* = 1$ .

Of course, if  $A < C$  and  $B = D$  then this situation

implies that management strategy has less gain than non-management when the other developer executes management strategy, or means the contrary strategy when the others abandon management strategy while the player finds that he will get severely punished due to increasing CPGRs. This means the player's strategy of choice depends on the other players' actions according to the seriousness of CPGRs. For example, after using non-management strategy, developers find themselves receiving less fines and the others playing management to the risk resource at the same time. Which choices will they make? Clearly, non-management strategy! In contrast, when they find they are receiving higher fines, the only right choice is to take up management strategy because if they give up management the nasty risk resources strategy within the others doing the same things. Thus  $F'(x_1^*) > 0$ ,  $F'(x_2^*) > 0$ ,  $F'(x_3^*) < 0$ ,  $x_3^*$  is the only ESS.

The result tells us the frequency  $x_3^*$  of developers out of all developers will manage the CPGRs after repeatedly playing the game, and the frequency playing non-management strategy will be  $(1-x_3^*)$ . Obviously, the frequency of management strategy players is rising with " $B - D$ " increasing, but increasing " $C - A$ " attracts some bad implications.

From the above analysis, we know that projects receiving little CPGR is possible if we reduce the expected benefit of non-management strategy over management strategy. Therefore, serious policy is necessary from government. In the next section, we discuss game strategies between monitors from environment protection departments (EPD) and CPGR-developers.

### 3. The Evolutionary Game Based Model of CPGR-Developers and EPDs

The developers have two choices: implementing either a management or non-management strategy in confronting CPGRs; meanwhile the EPDs can also choose between a monitoring or non-monitoring attitude. Through environmental monitoring, EPDs will provide judgment on whether CPGRs are acceptable, in which case no action is taken, or unacceptable, in which case a fine will be adjudicated. However monitoring needs funding and may even cost more than is bargained, thus creating in itself a problem for EPDs. In contrasting the comparative gain between various strategies, we assume the benefit to two players is 0 (in reality, the benefit is not 0) under the criterion that developers will execute a management strategy toward CPGRs and the EPD carries out non-monitor strategy. We then let  $C_1 > 0$  refer to the EPD's monitoring costs of CPGRs,  $C_2 > 0$  the punishment to EPDs for breach of duty from government,  $S > 0$  the fine to be imposed by EPDs on non-management behaviour of

firms, and  $R \geq 0$  the incentives or bonuses to be awarded from government to EPDs for good monitoring behaviours.

The payoff matrix for EPDs and CPGR-developers is shown in **Table 2**. EPDs and CPGR-developer are assumed to be bounded rational agents. We define  $x$  as the frequency of management strategy in the developer population and define  $y$  as the frequency of monitoring strategy in the EPD population. We also define  $u_{1t}$  as the expected gain for a management strategy,  $u_{1f}$  as the expected gain for non-management strategy,  $u_1$  as the average gain for all construction firms at a given generation,  $u_{2t}$  is the expected gain for monitoring strategy,  $u_{2f}$  is the expected gain for non-monitoring strategy,  $u_2$  is the average gain for all EPDs at a given generation.

The replicator dynamic equation for developers and EPDs are respectively expressed as Equations (5) and (6) as following:

$$F(x) = dx/dt = x(1-x)(S-A-Sy) \quad (5)$$

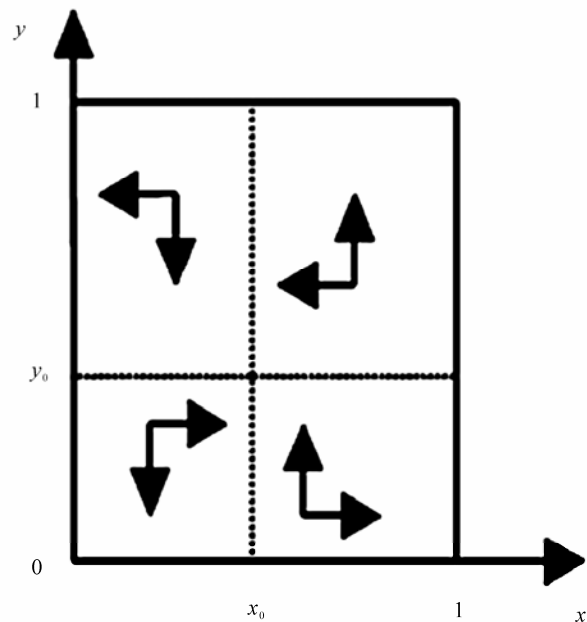
$$G(y) = dy/dt = y(1-y)[C_1 - C_2 - R + (C_2 + R)x] \quad (6)$$

There are two situations to be considered for developers according to Equation (5). One is that if  $y = (S-A)/S$  ( $S \geq A$ ), thus  $F(x^*)$  is 0, implying all  $x$  are trade-off solutions, and another is that if  $y \neq (S-A)/S$  then  $x_1^* = 0$  and  $x_2^* = 1$  while if  $y > (S-A)/S$  the solution is  $x_1^* = 0$  and if  $y < (S-A)/S$  then  $x_2^* = 1$ . Accordingly, there are two situations to be considered for EPDs. One is that if  $x = (R+C_2-C_1)/(C_2+R)$  and  $(R+C_2 = C_1)$ , then  $G(y) = 0$  implying all  $y$  are trade-off solutions, and another is that if  $x \neq (R+C_2-C_1)/(C_2+R)$ , then  $y_1^* = 0$  and  $y_2^* = 1$ , and when  $R+C_2 \geq C_1$  ( $x < (R+C_2-C_1)/(C_2+R)$ ), then  $y_1^* = 0$  otherwise If  $y_2^* = 1$ , and when  $R+C_2 < C_1$  ( $C_1 - C_2 - R + (C_2 + R)x > 0$ ) then  $G'(y_1^*) > 0, G'(y_2^*) < 0$  and  $y_2^* = 1$ . If denoting  $x_0 = (R+C_2-C_1)/(C_2+R)$ ,  $y_0 = (S-A)/S$ , and  $x_0 > 0$ ,  $y_0 > 0$ , we can understand the changing evolutionary ratios of developers and of EPDs (**Figures 2-5**).

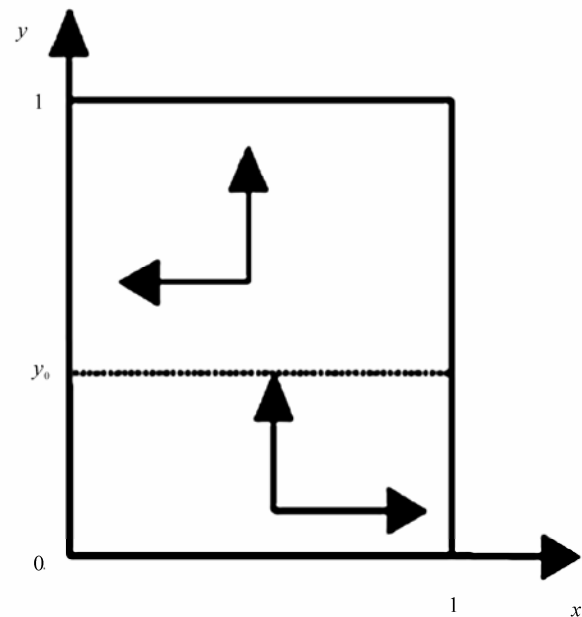
Just as depicted in **Figure 2** the strategies of EPDs and developers mainly depends on each other's choices of strategies, and further analyses made about **Figure 3** and **Figure 5** illustrates whether the fine is high or low the

**Table 2. Payoff matrix by EPD and PGR-developers.**

		EPD	
		No-monitoring	Monitoring
CPGR-Developer	Manag.	0, 0	0, -C <sub>1</sub>
	Non-manag.	A, -C <sub>2</sub>	A-S, R-C <sub>1</sub>



**Figure 2.**  $S > A, R + C_2 > C_1$ .



**Figure 3.**  $S > A, R + C_2 < C_1$ .

trade-off of ESS between EPDs and developers is  $x^* = 0$ ,  $y^* = 1$ . It means that the situation will arise when EPDs find higher monitoring costs with low incentives or rewards from government, or alternatively, it will occur when developers receive only small punishment with more green risk resources discovered by EPDs. Once achievement of game evolutionary, EPDs have to accept the non-monitor strategy and the developers accordingly take

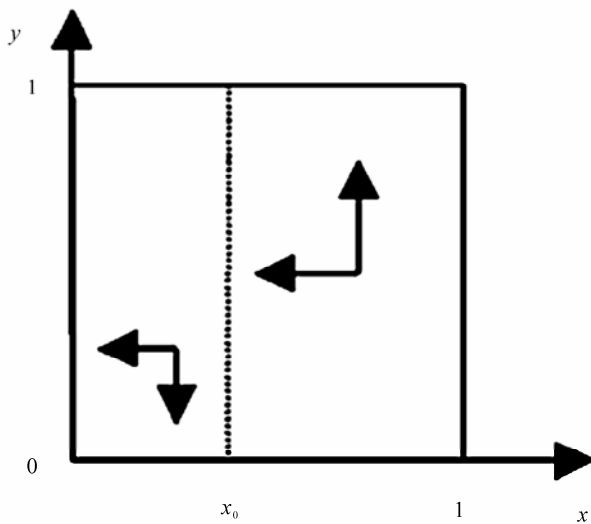


Figure 4.  $S < A, R + C_2 > C_1$ .

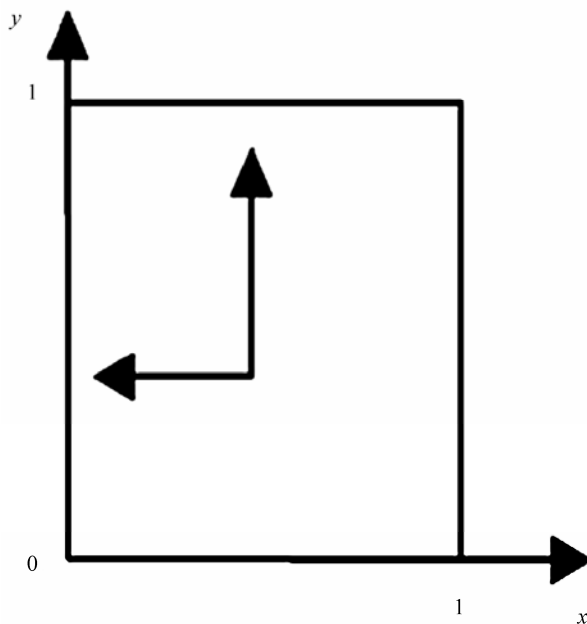


Figure 5.  $S < A, R + C_2 < C_1$ .

actions of non-management strategy. However, from **Figure 4** if  $x^* = 0, y^* = 0$  which indicates that CPGR-developers will ignore green risks regardless of the monitoring of EPD because the fines from authorities are far from non-management's interest.

#### 4. Conclusions and Suggestions

We can draw some conclusions from above analyses as follows:

- 1) Laws should be enacted to impose severe penalties

on any non-management firm and its executives, thereby increasing the cost if a non-management strategy is followed;

- 2) In order to enhance environmental activism, government should exact strong punishment on those EPDs failing in their monitoring duties, and promote active environment protection enforcement;

- 3) The government should cover the costs of EPDs and raise incentives and rewards by investing in them and supplying new equipment. Such policies will accelerate uncovering non-complying developers;

- 4) New government policies mandating changes in the management mechanism of EPDs to function more easily, efficiently and profitably, will reduce reporting and accountability costs. Such policies would encourage EPDs to monitor CPGRs more closely and successfully.

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# Green Inefficiency for Regions in China

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## ABSTRACT

*We used the directional output distance function to derive estimates of green inefficiency, shadow prices, and waste costs of three wastes (waste water, waste gas, and solid waste) for thirty regions in China during the 1996-2003 periods. There is an upward trend in green inefficiency in Chinese regions from 1996 to 2003. The green inefficiency score in west area is the lowest but in central and east areas are higher. The costs of wastes have an upward trend in east and central areas but downward trend in west area in the last two observation years.*

**Keywords:** Green Inefficiency, Environment Waste, Directional Output Distance Function, Shadow Price, China

## 1. Introduction

In the end of 1980's, China recognized Japan's Industrial development policy which supported Japanese small-sized industries after WWII, and Japan had become the 2<sup>nd</sup> biggest economic position in the world. Therefore, China introduced this policy to achieve its target of expediting domestic economy development.

The industries of mechanical, electronic, petrolic and chemical have been recognized as the most important industries in order to improve China's economy. Nevertheless, these industries also cause the problems of resources depletion and environment pollution. In the meantime, these industries have been developed quickly, and the conflict has become worst due to the lacking restriction against over resources depletion and environment pollution since 1980's. Thus, China has suffered the three biggest environment wastes which are byproducts accompany with the production of industries: waste water, waste gas, and solid wastes (here after: three wastes).

Water using and management has become a popular issue. The waste water is an important issue in China [1]. Cities in China discharging 10 billion tons of waste water every year. Pesticides in drinking water have also been a problem to the health of a community [2]. In 2005 the chemical firm of Jilin exploded and 100 tons of benzene flowed into Songhua River, then, had the water supply system of Harbin shutdown [3].

The main source of industrial waste gas emission is

one of the main sources of air pollution. Most of the existing analyses of air pollution abatement focus on its benefit evaluation [4,5], its possible impacts on economic activities [6], strategies to achieve it [7], or discuss the indices to measure the air quality [8]. Economically efficient abatements of air, solid, and waste pollution still receive relatively not much attention [9].

The investigation of industrial solid waste management in several countries is getting more important recently. For case studies of industrial solid waste and recycling, Casares [10] investigated Asegra in Spain and Donnelly [11] researched the United States and Germany. Moreover, Hogland and Stenis [12] described a method of organization for an industrial solid waste management system in Sweden.

China confronts the three waste problems under the policy of energy-saving. There is no literature taking environment wastes into account to measure national green inefficiency in previously. In this study we try to find out how the environmental problem serious is and provide our suggestion to improve this situation.

## 2. Methods

We will describe the methods of estimating the shadow price, waste cost and evaluating the inefficient of each province in this section.

Suppose that each decision making unit (DMU) uses  $N$  inputs to produce  $M$  desirable and  $J$  undesirable outputs and the output set of production technology can be de-

defined as:

$$P(\mathbf{x}) = \{ (\mathbf{y}, \mathbf{u}) : \mathbf{x} \text{ can produce } (\mathbf{y}, \mathbf{u}) \} \quad (1)$$

The undesirable output is the byproduct of the production of the desirable output, we assume that desirable and undesirable outputs are null-joint outputs if

$$(\mathbf{y}, \mathbf{u}) \in P(\mathbf{x}), \text{ and } \mathbf{u} = 0 \text{ then } \mathbf{y} = 0 \quad (2)$$

Equation (2) means that if a desirable output is produced in a positive amount then some undesirable outputs also be produced.

Färe and Primont [13] defined the weak disposability of undesirable outputs as follows:

$$(\mathbf{y}, \mathbf{u}) \in P(\mathbf{x}), \text{ and } 0 \leq \theta \leq 1 \text{ imply } (\theta \mathbf{y}, \theta \mathbf{u}) \in P(\mathbf{x}) \quad (3)$$

Equation (3) describes that reduction of undesirable outputs can reduce desirable outputs simultaneously while maintaining the same level of inputs.

Färe and Grosskopf [14] defined the directional output distance function as follow:

$$\begin{aligned} \bar{D}_o(\mathbf{x}, \mathbf{y}, \mathbf{u}; \mathbf{g}_y, \mathbf{g}_u) \\ = \sup \{ \beta : (\mathbf{y} + \beta \mathbf{g}_y, \mathbf{u} - \beta \mathbf{g}_u) \in P(\mathbf{x}) \} \end{aligned} \quad (4)$$

Equation (5) searches for the largest feasible expansion of desirable output vector  $\mathbf{y}$  in the  $\mathbf{g}_y$  direction and the largest feasible reduction of undesirable output vector  $\mathbf{u}$  in the  $\mathbf{g}_u$  direction. Färe and Grosskopf (2005) provide a relationship between the revenue function and directional distance function. The revenue function is defined as:

$$R(\mathbf{x}, \mathbf{p}, \mathbf{r}) = \max \{ \mathbf{p}\mathbf{y} - \mathbf{r}\mathbf{u} : (\mathbf{y}, \mathbf{u}) \in P(\mathbf{x}) \} \quad (5)$$

where  $\mathbf{p} = (p_1, \dots, p_M)$  is the desirable output price vector and  $\mathbf{r} = (r_1, \dots, r_J)$  is the undesirable output price vector. Equation (5) is the largest feasible revenue that can be obtained from input  $\mathbf{x}$  and output price vectors  $\mathbf{p}$ ,  $\mathbf{r}$ .

If the output set is a closed, nonempty convex set then the directional output distance function can be obtained from the revenue function as:

$$\begin{aligned} \bar{D}_o(\mathbf{x}, \mathbf{y}, \mathbf{u}; \mathbf{g}_y, \mathbf{g}_u) \\ = \inf_{\mathbf{p}, \mathbf{r}} R(\mathbf{x}, \mathbf{p}, \mathbf{r}) - (\mathbf{p}\mathbf{y} - \mathbf{r}\mathbf{u}) / \mathbf{p}\mathbf{g}_y + \mathbf{r}\mathbf{g}_u \end{aligned} \quad (6)$$

We can compute the price of the  $j$ 'th undesirable output as:

$$r_j = -p_m \left[ \partial \bar{D}_o(\cdot) / \partial u_j \right] / \left[ \partial \bar{D}_o(\cdot) / \partial y_m \right] \quad (7)$$

After measuring the shadow price from the above equations, we need alternative method, the directional

output distance function, to get the inefficiency value. The linear programming problem for each observation  $k$  as follow:

$$\begin{aligned} \bar{D}_o(\mathbf{x}^k, \mathbf{u}^k, \mathbf{y}^k; \mathbf{g}_u, \mathbf{g}_y) &= \max_{\beta, z_1, \dots, z_K} \beta \\ \text{s.t.} \\ 1) \sum_{k=1}^K z_k y_{km} &\geq y_m + \beta g_{ym}, \quad m = 1, \dots, M \\ 2) \sum_{k=1}^K z_k u_{kj} &= u_j - \beta g_{uj}, \quad j = 1, \dots, J \\ 3) \sum_{k=1}^K z_k x_{kn} &\leq x_n, \quad n = 1, \dots, N \\ 4) \sum_{k=1}^K z_k &= 1, \quad z_k \geq 0, \quad k = 1, \dots, K \end{aligned} \quad (8)$$

The  $\beta$  is the inefficiency value for observation firm. In this study, we use this directional output distance function to derive the estimates of shadow price, measure the inefficient values and take the shadow prices to compute the waste costs.

### 3. Results and Discussion

The major target of an economy is improving living standard in economic development processing. The GDP can be presented as living standard level, so we take GDP as desirable output. In order to increase the GDP that there have to cause some pollutions such as three wastes. We want to increase the GDP but not much pollution. These three wastes are unavoidable when the economy increases the GDP. Therefore, we call three wastes as undesirable outputs.

Data of the desirable output: GDP, be deflated to 1996 values, in each region is collected respectively as stated previously. Real capital stocks in 1996 prices are constructed based on Li's method [15].<sup>1</sup> The data of regional labor employment are established from the *China Statistical Yearbook*. The thirty regions are categorized into three areas. The three areas are the east area (abbreviated as 'E'), the central area (abbreviated as 'C'), and the west area (abbreviated as 'W').<sup>2</sup>

From *China Statistical Yearbook*, we establish a dataset for 30 regions in China (27 provinces and 3 munici-

<sup>1</sup>The capital stock data are not available in the *China Statistical Yearbook*. In this study, every regional capital stock in a specific year is calculated by the authors according to this formula: capital stock in the previous year + capital formation in the current year - capital depreciation in the current year. All the nominal values are deflated in 1995 prices before summations and deductions. We find the initial capital stock (capital stock data in 1995) from the research of Li (2003).

<sup>2</sup>According to the Grand Western Development Program, Inner Mongolia and Guangxi are included in west area.

palties) during 1996 to 2003.<sup>3</sup> There are one desirable output, three undesirable outputs and two inputs in our directional output distance function model. The values of monetary output and input are in 1996 prices.

Descriptive statistics for the outputs and inputs are provided in **Table 1**. From this table we know that desirable and undesirable outputs satisfy the assumption of null-joint outputs. All of the outputs are greater than zero and hence the directional output distance function can be applied to estimate the inefficiency value.

As **Figure 1** illustrates, there is an upward trend in inefficiency of annual average from 1996 to 2003. The best average efficiency occurred in 1996, after which inefficiency trends upward. During the Ninth-Five Plan, the inefficiency value went up in 1997 because of Asian Financial Crisis. The State Council of the People's Republic of China wanted to reduce the damage, they got an idea for increasing the Interior-Need and issued bond of 100 billion RMB to improve the economic situation in 1998. The policy reforms of China took place in this period that changed the inefficiency value across time.

The inefficient value decreased in 2003. We presume that the people prefer the economic growth of China because of the BRIC thesis. It expected the developing countries include Brazil, Russia, India, and China will become the biggest and fastest growing emerging markets.

There is an upward trend in inefficiency in east and west areas from 1996 to 2003. The inefficiency in central area gets down from 2002. Half of the most seriously pollution cities of the world are in the center area.<sup>4</sup> In average, the green efficiency in center area is the lowest. Relative to center area, there is the most efficient in east area.

We normalized the data, because of convergence problems, by dividing each output and each input by its mean value before estimating Equation (14).

The parameter estimates for the quadratic form of the directional distance function are provided in **Table 2**. Using these parameters and Equation (11) can measure the shadow prices of three wastes. The opportunity cost can be estimated by shadow price.

**Table 3** provides the estimates for the shadow prices and the costs of wastes. The cost is product of shadow price and waste quantity. The average shadow price of waste gas is the highest and the average cost of water waste has the highest level in sample periods. However, the average shadow price and average cost of solid waste are the lowest.

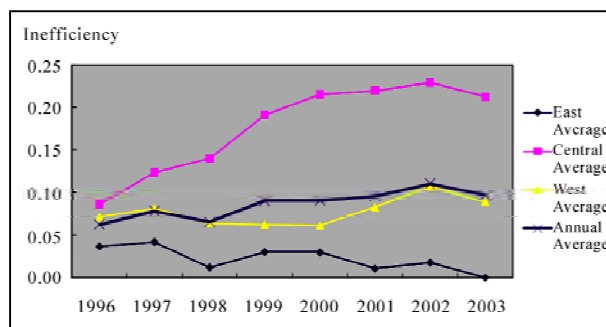
China should pay more attention to waste water which has the highest cost but not the highest shadow price.

<sup>3</sup>Chongqing became a municipality out of Sichuan in 1997 and in this study its outputs and inputs are included in Sichuan.

<sup>4</sup>These cities are Jilin, Shanxi, Henan, and Hubei.

**Table 1. Describing statistics for the outputs and inputs. (Base year: 1996).**

Variable	Mean	Standard deviation	Minimum	Maximum
Desirable Output				
Gross Domestic Product (million RMB)	235784	183387	6476	780181
Undesirable Outputs				
Volume of Industrial Waste Water (10,000 tons)	64761.54	51425.24	612.00	247524.00
Volume of Industrial Waste Gas (10,000 tons)	4680.23	3311.35	10.00	16139.00
Volume of Industrial Solid Wastes (10,000 tons)	2707.54	2145.65	1.00	9252.00
Inputs				
Capital Stock (million RMB)	1008847	838253	80454	3772421
Labor (10,000 persons)	2094.84	1520.93	117.70	6307.50



**Figure 1. Green inefficiency of annual average.**

This implies that the waste water causing high environmental damage but is relatively cheaper to deal with. Some provinces have made several policies to abate the waste water and these policies must be enforced persistently, in order to achieve the long run effects.

**Figure 2** to **Figure 4** show the total costs of waste water, waste gas and solid waste, respectively. The cost of three kinds of waste in east is the highest but in west is the lowest. From these figures we know that, the most important environmental problem is waste water which has the highest total cost. This is maybe the east area has many downstream rivers flowing into the Pacific Ocean. The east area has hence to pay more cost to deal with waste water.

In order to confront this environmental problem, China built a complete system about water resource management and water pollution control and revise Water Law of the People's Republic of China in 2002. But it didn't

**Table 2. Parameter estimates.**

Constant	-0.039
$\alpha_{input}$	0.45 0.448
$\beta_{D.O.}$	-0.703
$\gamma_{U.O.}$	0.273 -0.012 0.036
$\alpha_{input,input}$	-0.451 -0.040 -0.04 -0.573
$\beta_{D.O.,D.O.}$	0.017
$\gamma_{U.O.,U.O.}$	-0.057 0.154 -0.011 0.154 -0.24 0.035 -0.011 0.035 -0.042
$\delta_{input,D.O.}$	0.101 0.114
$\nu_{input,U.O.}$	-0.16 0.249 0.012 0.134 -0.022 0.002
$\mu_{D.O.,U.O.}$	0.086 -0.051 -0.018

Note: D.O. is desirable output. U.O. is undesirable output.

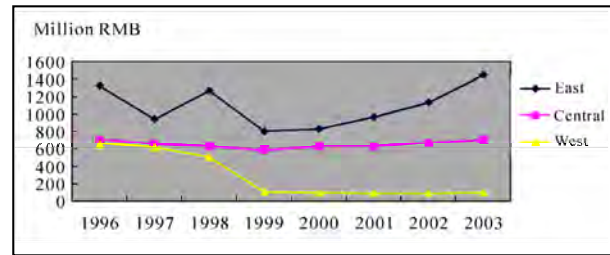
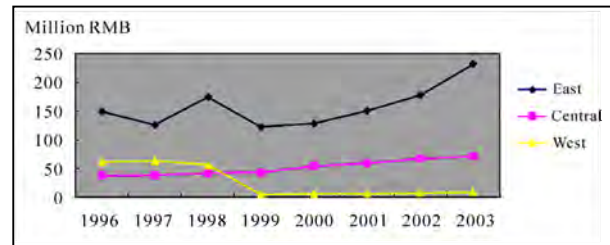
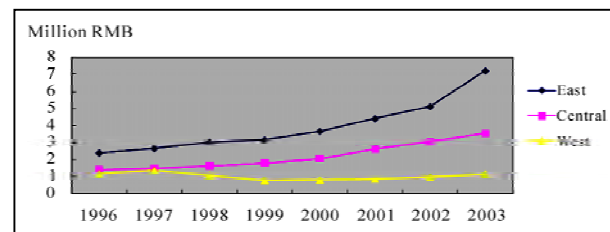
**Table 3. The estimates for the shadow prices and the costs of wastes.**

	Mean	Standard deviation	Minimum	Maximum
Shadow price				
Waste water	0.835	0.618	0.248	3.671
Waste gas	1.212	1.743	0.025	12.648
Solid waste	0.084	0.582	0.000	0.323
Cost of waste (Million RMB)				
Waste water	717.38	1097.98	2.35	8064.89
Waste gas	86.01	150.73	0.00	925.58
Solid waste	2.52	3.59	0.00	21.38

Note: Because  $\partial \bar{D}_o(\mathbf{x}, \mathbf{y}, \mathbf{u}; \mathbf{g}_o, \mathbf{g}_u) / \partial u_3 = 0$ , the estimate of shadow price in solid waste term equals zero.

reduce the cost immediately for waste water in 2003. In these figures we find that, the total cost of water waste is the highest. The water problem became a hot issue in every country in the world in this decade. It is costly to deal with the water waste no matter in developing or developed country.

**Figure 5** describes the total cost in three wastes in each regional average. We let the province in east area in the right, in west area in the left. The top-six waste cost in regional averages are: Jiangsu (E), Sichuan (W), Guang-

**Figure 2. The total cost of waste water.****Figure 3. Areas' annual total cost of waste gas.****Figure 4. Areas' annual total costs of solid waste.**

dong (E), Shandong (E), Henan (C) and Zhejiang (E). Most of east areas have higher waste cost. The lower waste cost in regional averages is: Tibet (W), Qinghai (W), Hainan (E), Ningxia (W), and Xinjiang (W). Most of west areas have lower waste cost. The average shadow price of three kind wastes in Jiangsu and Sichuan with the highest level especial in waste gas. Therefore, there is the seriously waste cost in these provinces. There is the lowest quantity average of three kind wastes especial in waste gas and solid waste, so the lowest waste cost in Tibet.

**Figure 6** presents the inefficiency in each regional average. The most efficient regions during the 1996-2003 periods are: Tibet (W), Tianjin (E), Zhejiang (E), Hebei (E) and Shanghai (E). The most inefficient regions during the observation periods as follow: Hubei (C), Henan (C), Hunan (C), Guangdong (E) and Guangxi (W). We find that, most of provinces with higher inefficiency value almost are heavy industry cities.

The inefficiency in the three regions presents a significance difference.<sup>5</sup> The operating efficiency in east area is superior to other regions obviously. Even east

<sup>5</sup>The Kruskal-Wallis test has significance at the 1% level.

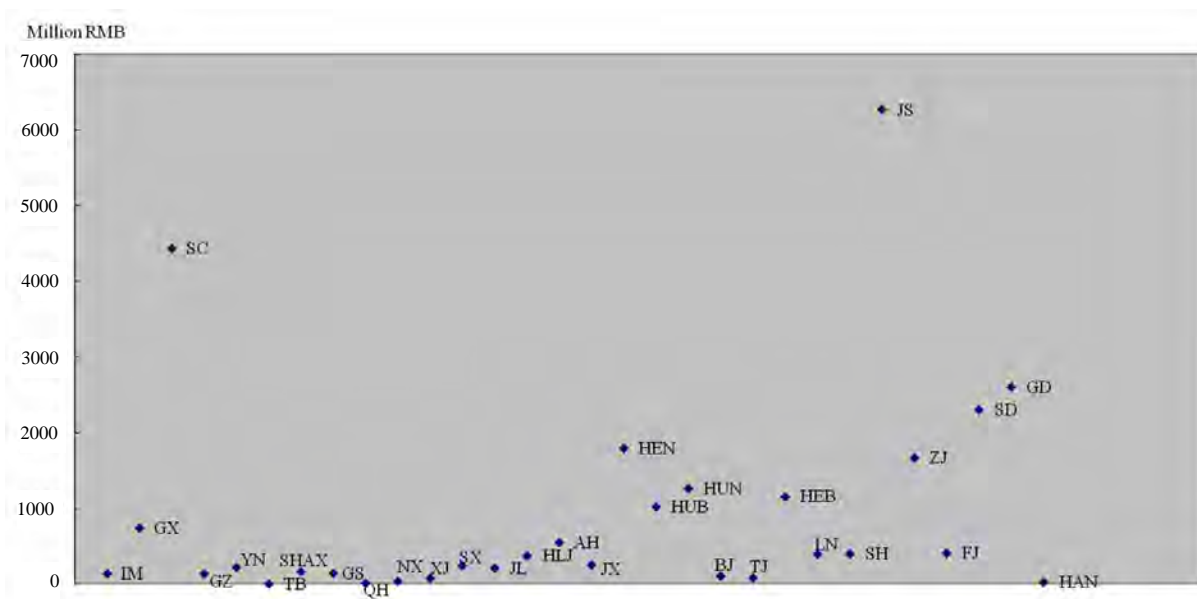


Figure 5. Regional averages of total waste cost.

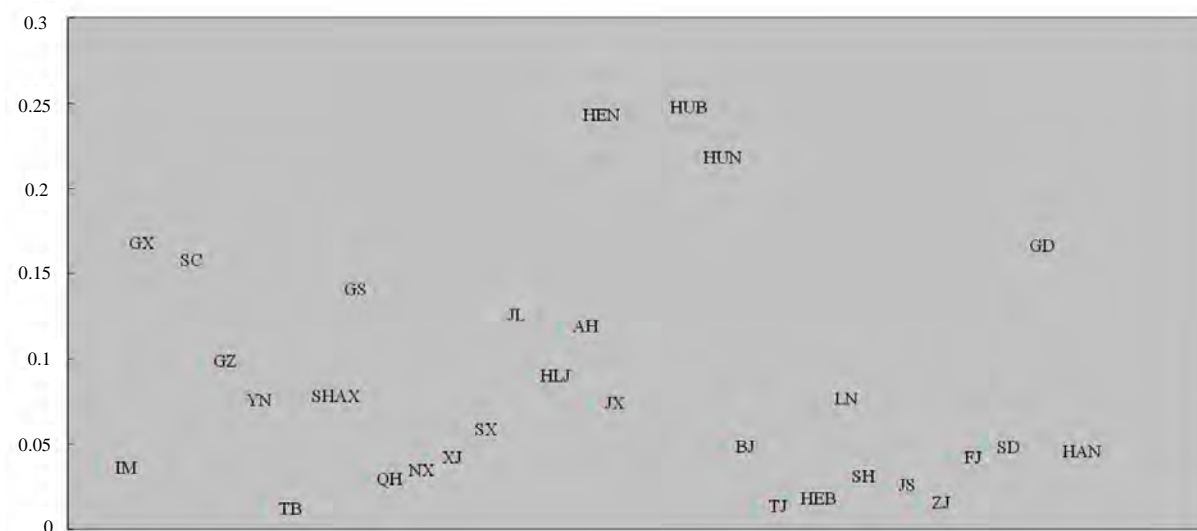


Figure 6. Regional averages of inefficiency.

area has higher waste quantity and cost. Development level plays an important role in our analysis process. The east area has the highest green efficient, because the input and output using is quite well in production procedure rather in other areas.

The inefficiency values of center area are higher than other areas during this study period obviously. The reform policy seems helpful to east area because of the lowest inefficiency value, but harmful to center area because it has constrained generation of three wastes to increase inefficiency value.

#### 4. Conclusions

Environmental protection is an important issues in recent. We built an observation set for 30 regions in China during 1996 to 2003. This set concluded one desirable output, three undesirable outputs, and two inputs.

This study used the directional output distance function to derive estimates of production inefficiency, shadow prices, and associated pollution costs of three wastes in China.

Our results indicate that an upward trend in inefficiency from 1996 to 2003. The value of inefficiency in

west area is the lowest but in central and east areas are higher in related. The costs of three wastes have an upward trend in east and central areas but downward trend in west area in the last two observation years.

We suggest that the China should abate wastes immediately to contract the cost of wastes to get better macro-economic performance.

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## Appendix

Abbreviation in provinces.

Province	Abbreviation	Area
Beijing	BJ	E
Tianjin	TJ	E
Hebei	HEB	E
Shanxi	SX	C
Inner Mongolia	IM	W
Liaoning	LN	E
Jilin	JL	C
Heilongjiang	HLJ	C
Shanghai	SH	E
Jiangsu	JS	E
Zhejiang	ZJ	E
Anhui	AH	C
Fujian	FJ	E
Jiangxi	JX	C
Shandong	SD	E
Henan	HEN	C
Hubei	HUB	C
Hunan	HUN	C
Guangdong	GD	E
Guangxi	GX	W
Hainan	HAN	E
Sichuan	SC	W
Guizhou	GZ	W
Yunnan	YN	W
Tibet	TB	W
Shaanxi	SHAX	W
Gansu	GS	W
Qinghai	QH	W
Ningxia	NX	W
Xinjiang	XJ	W





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- Modeling and measuring of water pollution
- New water purification technologies
- Purification of drinking-water supplies
- Radiation safety in atomic industry
- Risk assessment of contaminated environments
- Solid waste management
- Source apportionment of air pollutants
- Sources of air pollution
- Sustainable development of environment and ecosystem
- Treatment, disposal and discharge of wastewater
- Urban/indoor air pollution and control
- Water resources and quality assessment
- Water resource protection and sustainable use

We are also interested in short papers (letters) that clearly address a specific problem, and short survey or position papers that sketch the results or problems on a specific topic. Authors of selected short papers would be invited to write a regular paper on the same topic for future issues of the **JEP**.

### Notes for Intending Authors

Submitted papers should not have been previously published nor be currently under consideration for publication elsewhere. Paper submission will be handled electronically through the website. All papers are refereed through a peer review process. For more details about the submissions, please access the website.

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