CONTENTS

PURE HUMIC SUBSTANCES HAVE THE POTENTIAL

TO ACT AS XENOBIOTIC CHEMICALS – A REVIEW

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SUMMARY

In this review we argue against two paradigms: (1) humic substances (HS) are not taken up by aquatic organisms; (2) HS are inert in aquatic systems, except for the release of reactive oxygen species after irradiation. In fact, these paradigms are recycled and do not apply any longer. We show that HS-like substances, such as caffeic acid oxidation products, are taken up by all aquatic organisms studied so far. Furthermore, we present phenomenological as well as mechanistic evidence that HS have direct effects on aquatic plants and animals. The effects may be categorized as non-specific, such as expression of heat shock proteins (hsp) and modulation of biotransformation enzymes, or specific, such as inhibition of photosynthetic oxygen release in plants. Basic ecotoxicological requirements are fulfilled: several mechanisms apply to a variety of aquatic organisms, dose-response relationships and quantitative structure effect relationships may be established where applicable. We conclude that HS are natural xenobiotics that exert a chemical stress and, thus, are able to structure aquatic guilds by various modes of action.

KEYWORDS: Humic substances, [^{14}C]KOP, uptake, direct effects, heat shock proteins, biotransformation, inhibition of photosynthetic oxygen release.

1 INTRODUCTION

In all aquatic ecosystems, humic substances (HS) are the major component of dissolved organic matter (DOM) and exceed carbon in all living organisms by one order of magnitude or more [1–4]. Depending on the source of organic material involved in the humification process, HS comprise a variety of molecular structures and differ with respect to their origin. Microbial and abiotic degradation yields in a variety of molecular structures, such as alkylaromatic, quinoide, and aliphatic structures in the core, and amino acid or carbohydrate structures, and carbonyl-, carboxyl-, phenyl-, and hydroxyl groups in the periphery [4–6].

The ecochemical relevance of HS is mostly discussed with respect to their capability to bind or integrate pollutants like organic xenobiotics and heavy metals, consequently decreasing their bioavailability and toxicity [7–12]. Direct adverse effects are seldomly discussed. The functional groups responsible for the interaction with pollutants may also directly interact with biological systems, such as membrane affection by either accumulation [13] or tenside-like action of HS [14, 15]; alteration of reproduction of the nematode *Caenorhabditis elegans* [16]; effect on survival of the waterflea *Daphnia magna* [17]; influence on growth of terrestrial plants [18, 19]; modulation of enzyme activities [3, 19, 20]; and antimicrobial and antimycotic activity [22, 23], leading to suppression of pathogens. In addition, photochemical reactions can initiate reactions leading to reactive oxygen species, such as $^1O_2$, $O_2^{\cdot-}$, and H_2O_2 [24–26], which are able to induce oxidative damage to biomembranes. Alterations of oxygen-stress enzymes are caused by HS in the aquatic macrophyte *Ceratophyllum demersum* [27].

2 UPTAKE OF A HUMIC ACID LIKE SUBSTANCE

The question as to whether or not HS are taken up by organisms, has been argued intensively in the literature. One cannot deny that beneficial, as well as adverse effects can be observed when organisms come into contact with HS in their various forms: soil water solutions, peats, bogs, or HS-rich surface waters. Most soil scientists, for instance, attribute any effect on plants to indirect modes

of action, such as modulations of the bioavailability of key nutrients. So do many freshwater ecologists. In addition to altered bioavailability of nutrients, further indirect modes of action are discussed, such as decreased light climates which affect primary producers and optical foragers alike, altered food web structures, and decreasing space for water breathing organisms due to increasing anoxia in the lower strata of the water column [28, 29]. Most freshwater ecologists exclude direct interactions between HS and aquatic organisms, because uptake of HS is not considered to be feasible. In contrast, biomedical scientists accept uptake of HS up to approximately 1.0 kDa [30, 31]. Furthermore, they report interactions of HS with several receptors [30] and with blood coagulation [32]. Investigations with radiolabeled humic acid-like substances show that 1-5% of topically applied humic polymers penetrate from an 1% W/O emulsion into human skin [33].

Few earlier studies [32, 34] discuss the uptake as a decisive basic mechanism to explain surprising results, such as modulation of photosynthesis in macrophytes and algae, induction of heat shock proteins (hsp) in fish and invertebrates, modulation of transformation enzymes, and alteration of the endoplasmic reticulum [35]. In one key study, Ziechmann [20] writes that humic acid precursors, namely, from aqueous peat extracts and compressed peat, pass through the skin of pigs and mice and can accumulate subcutaneously. Recent studies [36] also show that HS or at least parts thereof can be taken up by cell cultures and, at least, parts of these molecules can be found even in the DNA. Nardi [19] showed that the physiological effects of HS on terrestrial plants depends on the source, concentra-tion, and molecular mass of the HS. The authors present evidence that HS <3.5 kDa easily reach the plasmalemma of higher plant cells and, in part, are taken up. Thus, the uptake of HS by organisms can be counted as a direct effect, although mechanistic studies are rare.

In an unpublished study, we present evidence that ^{14}C-labeled humic-like substances prepared by enzymatic oxidation of caffeic acid with tyrosinase (EC 1.14.18.1) [37] are taken up and bioconcentrated by several aquatic organisms. The peak molecular mass of the caffeic acid oxidation product (KOP) detected from a non-radioactive sample was found to be 11,600 Da [38]. Figure 1 shows that, after 24 hours of exposure, a macrophyte (*C. demersum*), an invertebrate (*Gammarus pulex*), and a vertebrate (tadpoles of the moor frog *Rana arvalis*) are able to bioconcentrate ^{14}C in their bodies. The percentage uptake of the exposed [^{14}C]KOP is: *C. demersum* 7.3 $\pm$ 1.4%, *G. pulex* 6.9 $\pm$ 1.0%, and *R. arvalis* 11.7 $\pm$ 2.7%. It may still be argued that it is not the intact oxidation product of caffeic acid, but also smaller photodegradation products, which are bioconcentrated. Nevertheless, it is evident that at least low-molecular mass (photodegradation) products of the humic-like substances are taken up and bioconcentrated by aquatic organisms, and that the bioconcentrated substances are responsible for several effects addressed below, which are feasible only if HS are themselves taken up by the aquatic organisms. If parts of the broad molecular mass distribution of caffeic oxidation products are in the molecular mass range of 1.0 kDa, they cover well the molecular masses of most fulvic acids (FA) in aquatic ecosystems [2].
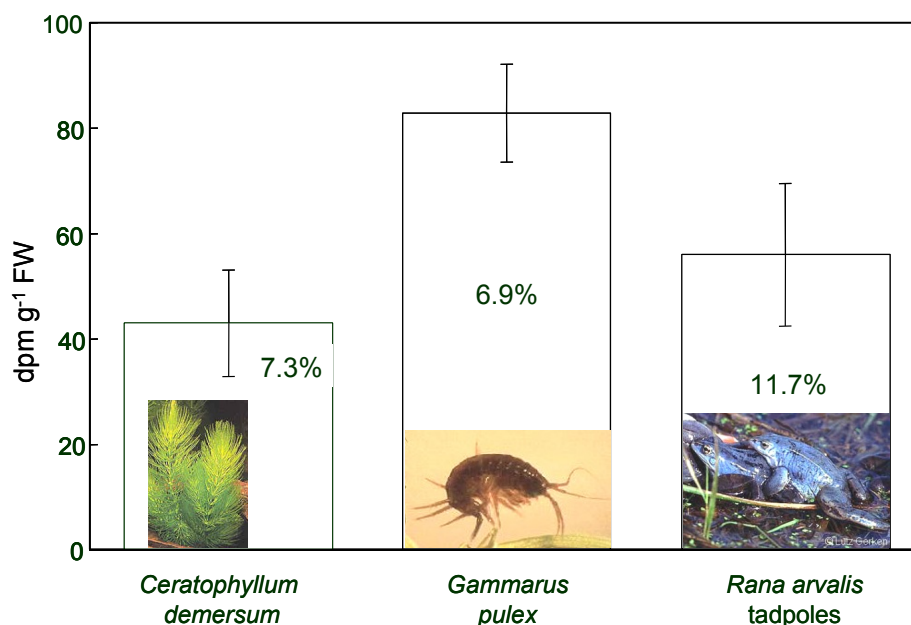
FIGURE 1 - Uptake of [^{14}C]-labeled caffeic acid oxidation product ([^{14}C]KOP) by three aquatic organisms within 24 hours (means $\pm$ standard deviation) (unpublished).
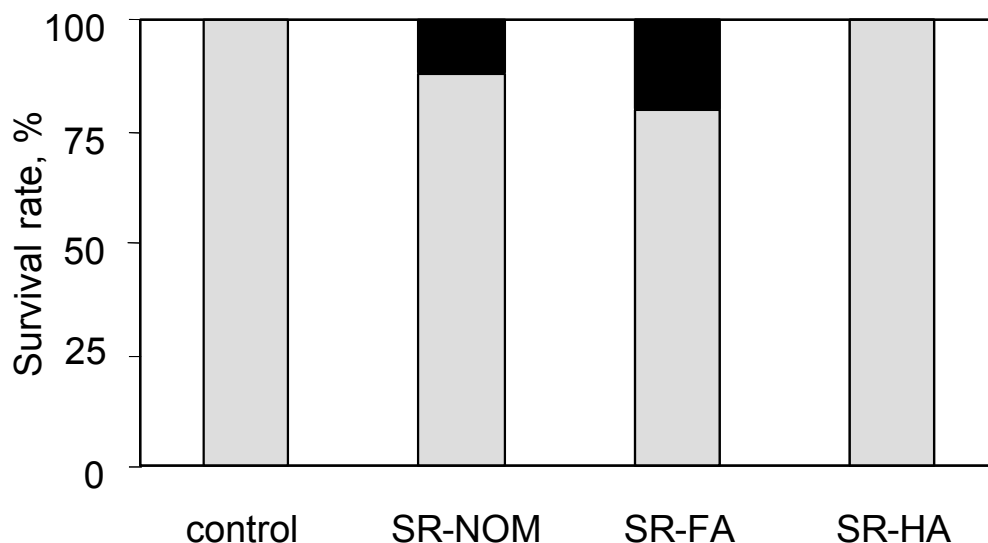
FIGURE 2
Survival rates of the common European freshwater snail, *Lymnea stagnalis*, exposed to 0.5 mg L^{-1} DOC of various HS
from the Suwannee River (SR) for 24 hours. Grey columns: living snails; black columns: dead snails (unpublished).

3 TOXIC EFFECTS

A. PHENOMENOLOGICAL EVIDENCE

Invertebrates: Snails

From recent cross experiments in which the European freshwater snail, *Lymnea stagnalis*, is exposed to different HS from the Suwannee River, clear evidence exists that under circum-neutral conditions the snails respond to HS exposure, as if exposed to man-made chemicals (xenobiotics). After a 24-h exposure to 0.5 mg L^{-1} DOC the activity of the transformation enzyme systems is extremely elevated, and Suwannee River natural organic matter (NOM) and FA, but not humic acids (HA), cause death of 10–20% of the animals (Figure 2). This finding means that HS appear to have a toxic potential per se.

Future studies may determine whether the toxic potential of HS pertains only to exotic species or also to indigenous species. Probably, the indigenous flora and fauna are better adapted to 'their' HS, than are exotic species.

Fish

In a recent study, Meinelt et al. [39] tested the survival of embryos of an *r*-strategist (zebrafish, *Danio rerio*), which were exposed to the synthetic HS1500 over a period of six weeks. Figure 3 shows that a concentration of 500 mg L^{-1} HS1500 (a clearly higher concentration than commonly found under natural conditions) significantly reduces the survival rate of the exposed embryos. On the oth-

er hand, low concentrations of 5 to 50 mg L^{-1} increased the survival rate relative to the control. But, since even under natural conditions a great proportion of embryos dies during ontogenesis, it cannot be judged whether the obvious decrease in the embryo mortality rate is a really beneficial effect or within natural ranges. Typically the offspring of *r*-strategists does not contain large amounts of energy reserves, whereas the number of the gametes produced by the parents is huge. Environmental changes will thus immediately lead to increased losses in the brood stock. Such high HS concentrations like 500 mg L^{-1} might be toxic to the weak juveniles.

On the other hand, the offspring of the so-called *K*-strategists is energetically better provided than that of the *r*-strategists. The „life-bearing" swordtails (*Xiphophorus helleri*) are typical *K*-strategists. Exposure of swordtail to HS1500 concentrations of up to 180 mg L^{-1} did not significantly increase the mortality of the brood stock over a five-month period (Fig. 4). In a long-term study, Meinelt et al. [39] did find a better growth of juveniles (length, mass, condition factor) as well as a better compensation of long-term stress (handling) in the HS-exposed groups. This means that, particularly at low HS concentrations, fish have more advantages than disadvantages. It can be speculated that this might be due to the inhibition of fish-pathogenic microbes like bacteria, fungi, and viruses. At the end, the question of whether or not HS are beneficial to fish is a function of the HS concentration, and may be also of the HS qualities, the energy content of the fish, and the inhibition of pathogens as well.
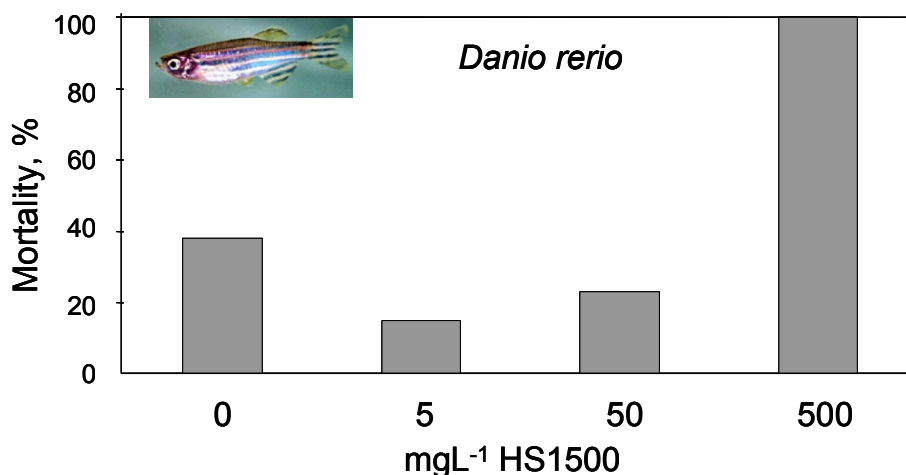
FIGURE 3 - Modulation of the mortality rate of embryos of a *r*-strategist (zebrafish, *Danio rerio*) exposed to different concentrations of the synthetic HS1500 [unpublished].
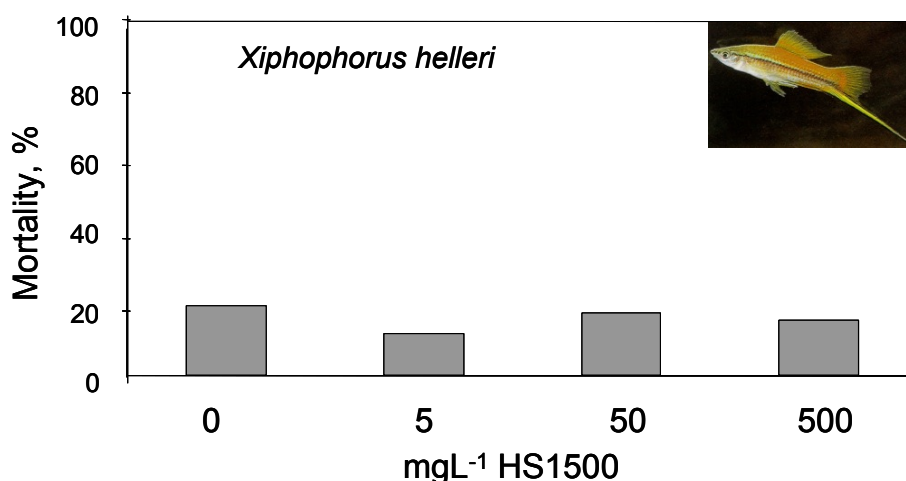
FIGURE 4 - Modulation of the mortality rate of embryos of a *K*-strategist (swordtail, *Xiphophorus helleri*) exposed to different concentrations of the synthetic HS1500 [39].

B. NON-SPECIFIC MECHANISMS

Non-specific mechanisms were studied by analyzing heat shock proteins (hsp) and biotransformation enzyme activity. Hsps are a chaperon family, protecting proteins in the cells during stress phases. Their cellular content increases after stress as caused by heat, heavy metals, and xenobiotics, with denaturated proteins being the causing principle [41]. Heat shock protein 70 (hsp70) binds to *de novo* synthesized proteins to prevent mistakes in folding, and in this form the protein is transported to its target place, where it achieves its final form and function. Hsp 70 also binds to partly denatured proteins to either refold them correctly and retain their function, or, if damage is too severe, guide them to places of controlled lysis.

Modulation of biotransformation enzymes, such as glutathione *S*-transferases (GSTs) is another unspecific physiological reaction of stressed organisms. GSTs are ubiquitous conjugation enzymes of the biotransformation pathway. They react with moderate hydrophilic xenobiotics, which include electrophilic groups. Activation by other enzyme systems like cytochrome P-450 monooxygenases is not required, if the substance already comprises

a functional group for the GSTs. Conjugation to glutathione increases water solubility of the substances and supports their excretion. A broad substrate specificity is attained by several soluble GST isoenzymes, and one microsomal form [40].

Heat shock protein 70

The expression of hsp 70 in carp shows different background intensities in the control fish, depending on the tissue: High expression in the liver, low to very weak expression in the gills, or the muscles, respectively. In the gills, which are the main contact organs during exposure *via* the medium, the moderate expression of the hsp70 is highly increased by all fractions of Suwannee River HS (Fig. 5A). The muscles show nearly no reaction on the HS (not shown). The different Suwannee River HS fractions (FA, HA, and NOM), caused almost no different reactions, only FA induced a slightly stronger reaction in the gills. Expression of hsp 70 after exposure of the carp to Svartberget, Humex A, Humex B, and Nordic Reference NOMs are investigated in gills only. All control carps show weak or no hsp 70 expression; hsp 70 are slightly increased by Svartberget and Nordic Reference NOMs and clearly increased by Humex A and Humex B NOMs (Fig. 5B).

The displayed mechanism does not only apply to fish, but seems to be more common. For instance, also gammarids show similar reactions upon exposure to HS isolates. In a screening test we exposed gammarids from Lake Müggelsee (Berlin, Germany) and Lake Baikal (Siberia, Russia) to Sanctuary Pond NOM (Ontario, Canada). Exposed individuals of *Gammarus tigrinus* and *Gammarus ischnus* expressed hsp 70 more strongly than control individuals (Fig. 5C). The induction of hsps appears to be a general reaction of aquatic organisms to exposure of HS and NOM.
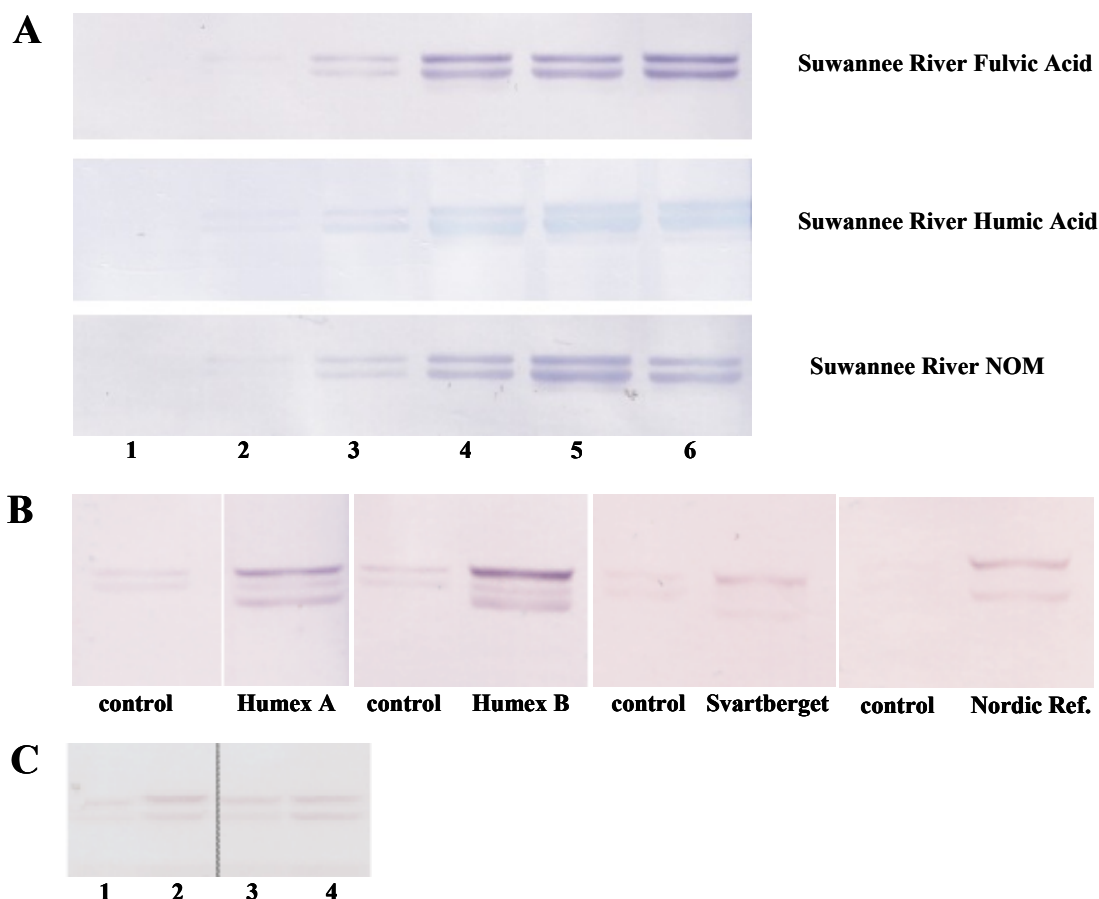
FIGURE 5 - **Western Blot detection of expression of the heat shock protein 70 in gills of carp, and in gammarids after exposure to HS. A: Carp gills, lane 1-3: control carp, lane 4-6 exposure to 5 mg L^{-1} isolated Suwannee River HS fractions, as indicated. Each line represents a sample of one single carp to show individual differences. B: Carp gills of control versus exposed carp. Exposure to 0.5 mg L^{-1} of the named NOMs from the Nordic Countries, from left to right: HUMEX A, HUMEX B, Nordic Reference, Svartberget, all NOM. C: Amphipods, exposed to 50 mg L^{-1} of Sanctuary Pond NOM lines 1, *Gammarus tigrinus* control, 2, *G. tigrinus* exposed, 3, *G. ischnus* control, 4, *G. ischnus* exposed. A detection of hsp 70 in the Baikalian *Eulimnogammarus cyaneus* failed (not shown) (from [42]).**
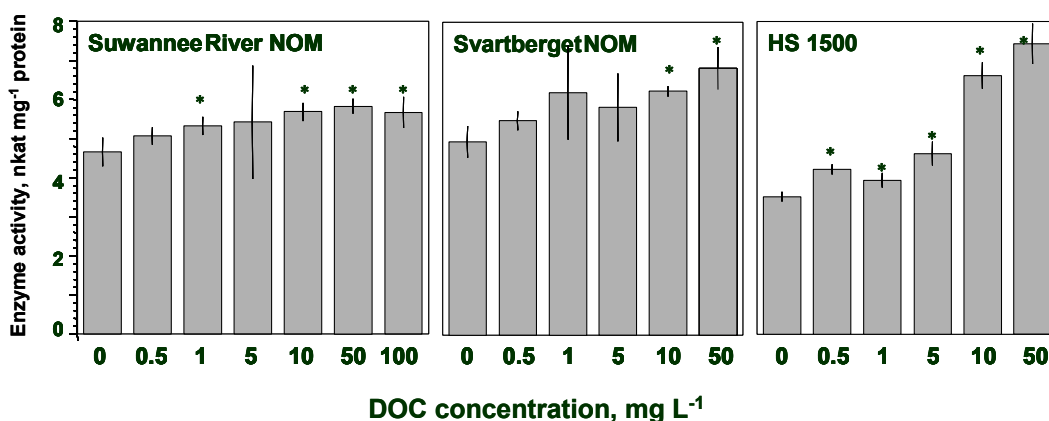
FIGURE 6
Glutathione-*S* transferase response in *Daphnia magna* with increasing concentrations of two NOM isolates
and of the synthetic HS1500 [42] (means, ± standard deviation) * significantly different from the control.

For these physiological interactions, obviously caused by HS, a fundamental requirement is that the HS come into contact with cell surface or cell internal structures. Expression of hsp 70 increases, if denatured proteins occur in the cell, no matter, if physical stress, like heat, or chemical stress, like xenobiotics, lead to their denaturation [43]. Thus, the used HS apparently cause protein denaturation in the gills of the carp and in the amphipods. A further possibility is the HS mediated generation of reactive compounds, which then will cause damages to the proteins. HS from different origin cause similar effects, but vary in intensity.

Dose-response: biotransformation enzymes (glutathione S-transferases)

In a study on *D. magna* Wiegand et al. [42] found that under pH neutral conditions HS isolates from the Suwannee River activate biotransformation enzymes such as glutathione-*S* transferases (GST). There is a dose-response relationship between HS and NOM exposure, and biotransformation enzyme activity (Fig. 6). It is evident that *D. magna* responds significantly to increasing exposure concentrations, with increases in GST activity. This is most pronounced with the synthetic HS1500 and least pronounced with Suwannee River NOM. Since all three isolates activate the phase II enzyme, a common mechanism might be assumed.

C. SPECIFIC MECHANISMS

Very recently, we tried to elucidate in more detail, the mechanism behind the HS- and NOM-induced modulation of photosynthetic oxygen release. The test alga was *Scenedesmus armatus*. Prior to photosynthesis measurement, the algae are exposed to HS and, in order to avoid light quenching during measurement, are transferred into a HS-free synthetic medium. Suwannee River NOM, a forest

soil leachate FA, and a synthetic HS (HS1500) significantly reduce the photosynthetic oxygen release (Fig. 7).[1] The reduction must be due to internal cell mechanisms, probably to interference of HS or their low-molecular mass fractions within the photosynthetic electron chain.
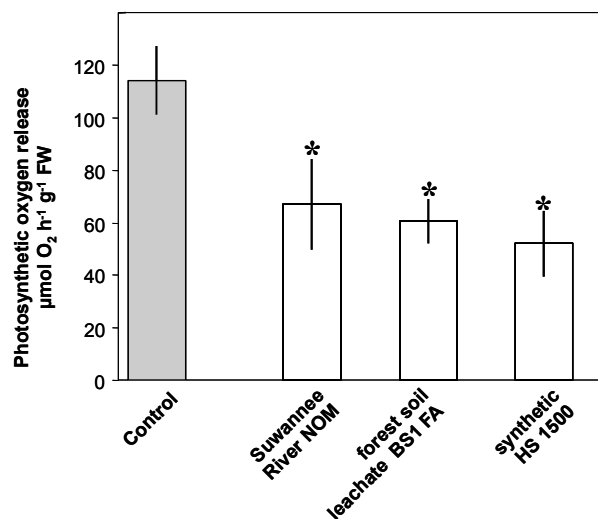
FIGURE 7 - Reduction of photosynthetic oxygen release in the coccal green alga *Scenedesmus armatus* after 18-h pre-exposure to 0.5 mg L^{-1} DOC of three HS and NOM isolates [47]. Data are means of three replicates, ± standard deviation; * significantly different from control; FW = fresh weight

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1   Perminova et al. [44] did not find any apparent effect of HS on the green algal species *Chlorella vulgaris*, as determined by chlorophyll fluorescence measurements. This finding, however, does not contradict the statement above for two reasons: first, the applied toxicological endpoints are of different susceptibility (according to our experience with *S. armatus*, chlorophyll concentration is not a very sensitive toxicity endpoint); second, 1 h of exposure time may generally be too short to provoke detectable changes.
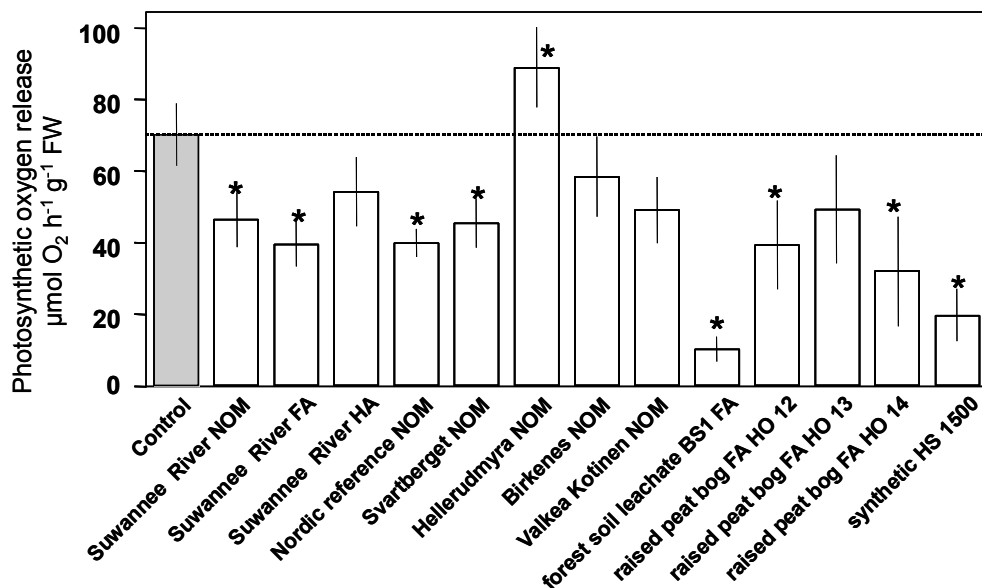
FIGURE 8 - Photosynthetic oxygen release in the coontail *Ceratophyllum demersum* after 24 h exposure to different HS and NOM, 0.5 mg L^{-1} C each. Prior to photosynthesis measurements, the plants are transferred into HS-free solutions. Most HS and NOM isolates significantly reduce the oxygen production, only one isolate significantly enhances it. Data are means of three replicates, $\pm$ standard deviation; * significantly different from the control; FW = fresh weight (modified from [47]).
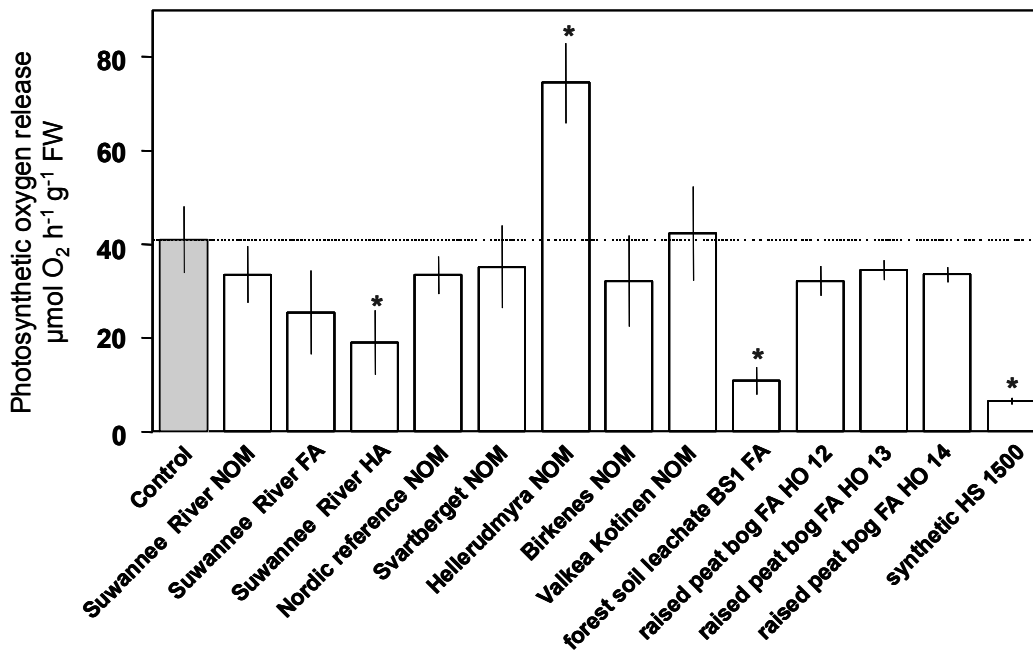
FIGURE 9 - Photosynthetic oxygen release in the tropical water moss *Vesicularia dubyana* after 24 h exposure to different HS and NOM, 0.5 mg L^{-1} C each. Prior to photosynthesis measurements, the plants are transferred into HS-free solutions. Three HS isolates significantly reduce the oxygen production, only one isolate significantly enhances it. Data are means of three replicates, $\pm$ standard deviation; * significantly different from the control; FW = fresh weight (modified from [47]).

Similar results, including hints on potential modes of action are obtained with two macrophytes, the hornwort *C. demersum* (Fig. 8) and the tropical water moss *Vesicularia dubyana* (Fig. 9). Recent microbiological studies show that HS have the potential to act as electron acceptors [45, 46], thus it appears most likely that this ability also applies to the effect described for algae.

Pflugmacher et al. [27, 47] describe the modulation of various physiological and biochemical parameters of *C. demersum* in the presence or absence of HS. In *C. demersum*, the photosynthetic oxygen release is significantly reduced by 8 out of 13 HS and NOM isolates, whereas in *V. dubyana* only 3 of 13 isolates reduce the oxygen release (Figs. 8, 9). The adverse effect of HS and NOM exposure can be seen even with the naked eye, for instance, *C. demersum* eventually turns yellow upon exposure to the forest soil leachate (BSI).

So far, the evidence that HS or NOM are the causative agents of direct effects on aquatic organisms remains somewhat circumstantial, because one cannot exclude that the observed effects may be due to contaminants sorbed onto the tested isolates. However, evidence would strongly increase, if the observed effects could be related to structural features of HS themselves, for instance by quantitative structure activity/effect relationships (QSAR). In fact, a QSAR can be established for the inhibition of photosynthetic oxygen release in aquatic plants. The electron trapping property can be attributed to the quinoide fraction of HS and NOM. Quinoide structures can act as electron acceptors, and thus interfere with the electron flow in photosystem I. Taking semiquinone radicals, which can be determined by electron spin resonance, as an indirect, but significant measure for quinoide structures [48–50], it is evident that the reduction of photosynthetic oxygen release can be significantly related to quinoide structural units in the HS materials (Fig. 10). The spin content of the HS and NOM predicts approximately 90% of the reduction of photosynthetic oxygen release in both macrophytes tested so far [51]. To date there is no mechanistic explanation for the different behavior of Hellerudmyra NOM (Figs. 8 and 9).
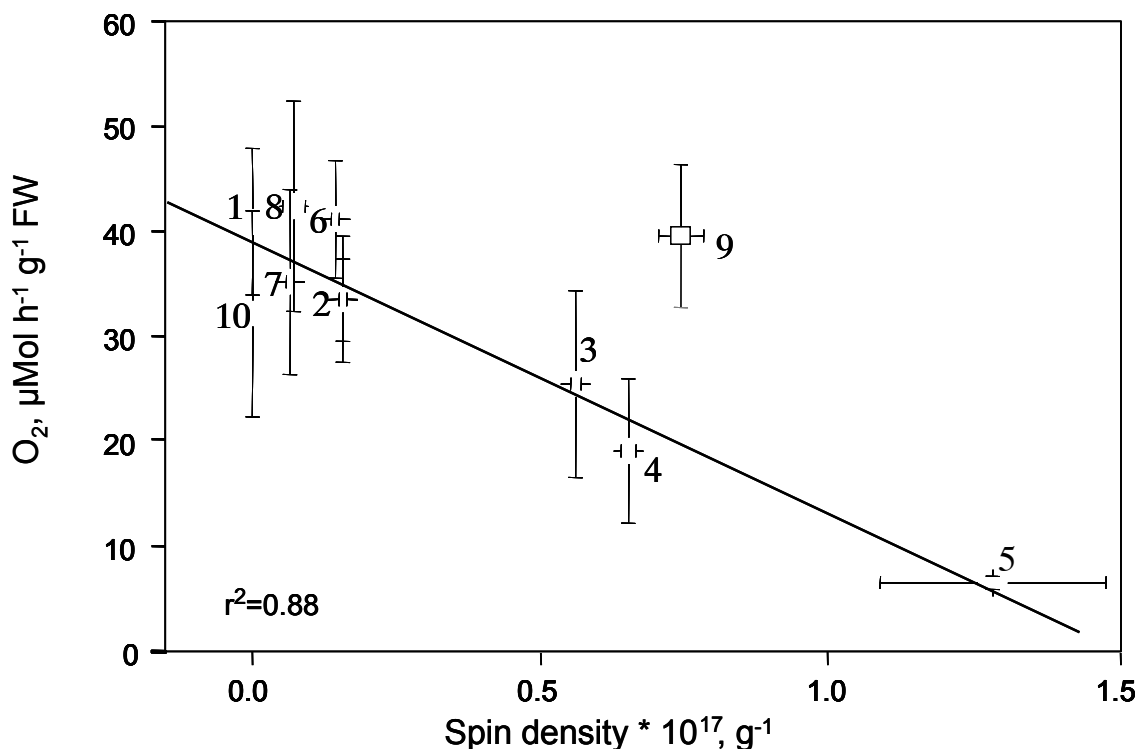
FIGURE 10 - Spin density of HS and NOM as a predictor for the reduction in photosynthetic oxygen release in *Vesicularia dubyana* [51]. 1: control; 2: Suwannee River NOM, 3: Suwannee River FA, 4: Suwannee River HA, 5: synthetic HS1500, 6: Hellerudmyra NOM, 7: Svartberget NOM, 8: Valkea-Kotinen NOM, 9: Hietajärvi NOM, 10: Birkenes NOM. Note that several soil and peat HS and NOM isolates which have not been tested with aquatic plants so far, possess even a higher spin content than the synthetic HS1500. Soil and peat HS have even higher spin contents than the displayed HS and NOM isolates. Hietajärvi NOM has been excluded from the regression.
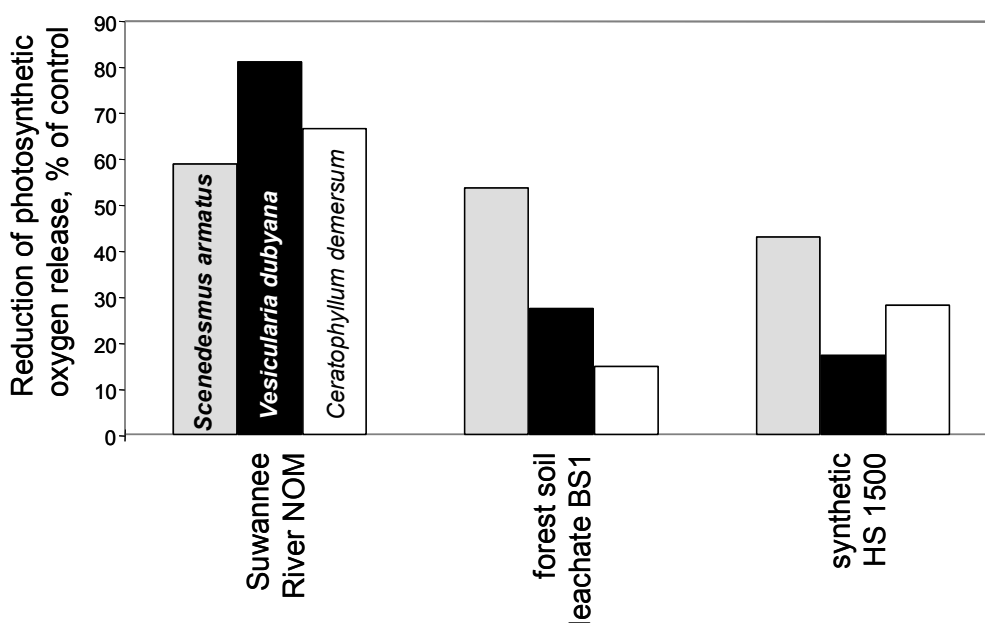
FIGURE 11 - Different susceptibilities of the three species of aquatic plants towards three different HS or NOM, as indicated by reduction of photosynthetic oxygen release [11].

When comparing all three aquatic plants tested so far (Fig. 11), it is evident that there is no 'most sensitive' species. With Suwannee River NOM, the most sensitive species is the coccal green alga *S. armatus*; with the soil FA it is the angiosperm *C. demersum*; and with the synthetic HS1500 it is the water moss *V. dubyana*. That means that a specific region, with specific terrestrial plant cover resulting in HS with specific chemical features, will produce a specific aquatic community under non-eutrophicated conditions. Some general rules may apply, such as water mosses gain dominance in humic waters. But, even his statement has to be confirmed in future studies. At present we are still clearly in the realm of information gathering, particularly since such effects of HS on photosynthetic oxygen release of aquatic plants are unexpected and contradict conventional paradigms on the inability of plants to take up HS or NOM.

CONCLUSION

The knowledge, of how HS affect exposed aquatic organisms, has been rather limited. In many instances, only indirect, external action like modulations of bioavailability of nutrients, bioavailability of metals, and xenobiotics, or the suppression of pathogens have been studied. In this review, we show that HS and NOM are able to directly affect physiological precesses of aquatic organisms comparable to xenobiotic chemicals. Upon HS exposure, aquatic organisms display induced hsp concentrations or altered activities of biotransformation enzymes,

leading mainly to reduced life times or survival. Furthermore, the numbers of offsprings of the nematode *C. elegans* may be altered, mainly increased [15]. With aquatic plants, HS and NOM may reduce photosynthetic oxygen release. The quantitative expression of these effects depends on the concentrations of quinoide structures in the humic materials. All the effects mean that populations, consortia, and communities (guilds) of aquatic organisms will be structured according to their susceptibility towards ambient HS. We may assume that with increasing numbers of ecological/ecochemical studies on HS, an increasing number of to date unexpected effects in freshwater ecosystems will be quantitatively related to specific structures in HS.

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MONITORING TSP, PM 10 AND PM 2.5 AT

A SEMI-REMOTE AREA IN NORTHERN ITALY -

RELATIONSHIPS BETWEEN PM 10 AND PM 2.5

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SUMMARY

Total Suspended Particulate (TSP), PM10 and PM2.5, concentrations were monitored over a period of 22 months (from March 2000 to December 2001) at the AIRMON station of the Joint Research Centre in Ispra, a semi-remote area in Northern Italy (ca. 70 km north of Milan). Monthly mean concentrations up to 90, 55 and 48 µg/m³ were measured for TSP, PM10 and PM2.5, respectively. The contribution of PM10 to the total particle mass (TSP) varied from 40% to 90%; with the highest contribution being found during the winter months. The PM2.5/PM10 ratio varied from 0.73 to 0.96, which indicates the high content of particles with diameter less than 2.5 µm in the PM10 fraction. Preliminary evidence indicates that by absence of local sources, long-range transport can significantly contribute to local air pollution with small particles.

KEYWORDS:
Particulate, long-range transport, TSP, PM10, PM2.5.

In this context, apart from the measurements of the classical air pollutants (NO_x, SO_2 etc.) ambient air measurements for particulate matter (PM10) are foreseen, in view of the limits to be in force by the year 2010. At that time already existing limit values in the Member States have to be replaced by the new EU limits. A 2 step approach is foreseen until January 2010, with the reduction of the limit values to 20 µg/m³ as annual mean and 50 µg/m³ as daily values (not to be exceeded 7 days per week), respectively.

The setting up of limit values by the EU was guided by the fact that exposure to particles and, especially, to those with an aerodynamic diameter less than 10 µm might have a direct impact on human health, since they can penetrate beyond the upper respiratory tract [2]. On contrary to the larger particles, fine particles have a relative longer residence time in the atmosphere and can, therefore, travel over long distances. Hence, air quality at regional and local level can significantly be influenced by long range transport of fine particles, in addition to emissions from local sources.

The present communication aims to contribute to the question of the importance of long range transport for particles and its impact on local air quality of an area, which can be considered as semi-remote and which is almost free of major local pollution sources.

INTRODUCTION

The increasing scientific and medical evidence to the health impact due to exposure to particulate matter is reflected in the recently approved EU directive on air quality [1]. Consequently, an evaluation of the ambient air concentrations of a number of priority air pollutants by the Member States will be required.

METHOD

According to the Community Directive 96/62/EC [3] on ambient air quality assessment and management and the Directive 1999/30/EC [1] relating to the establishment of limit values, the European Commission has mandated the European Normalisation Centre CEN to establish a reference method for the measure of PM10 and PM2.5.

The work on PM10 resulted in the standard EN 12341 describing the manual gravimetric method as reference method, while the work on PM2.5 has not finalised yet.

All particulate measurements (TSP, PM10, PM2.5) carried out at the AIRMON station from the year 2000 have been done by the manual gravimetric method described in the standard EN 12341 for PM10. The method consists of an inlet system, which provides the desired particle fraction, a quartz fibre filter on which the particles are collected, a sampling pump that can be regulated and a detailed procedure for the gravimetric analysis of the filters. The instruments used have been low volume samplers (Kleinfiltergeräte, LVS3) with specific sampling heads for TSP, PM10 and PM2.5. The particulate matter has been sampled for 24h with a volume of 2.3m^3/h. The mass of the particulates collected on the quartz fibre filters is determined by weighing the filter before and after the sampling time at defined climatic conditions.

RESULTS AND DISCUSSION

The monthly average concentrations of TSP, PM10 and PM 2.5 from March 2000 to December 2001 are presented in Figure 1. TSP concentrations varied from a low

of 27 µg/ m³ to a high of 90 µg/ m³. In the months May and June 2000, TSP concentrations were exceptionally high compared to the values measured during the same time period in 2001 (35 µg/ m³ to 42 µg/ m³). The annual average concentration for TSP at the Ispra monitoring station, calculated from the monthly average values, was found to be 48 µg/ m³. This corresponds to total particulate, without any separation into primary and secondary particle contribution. Interestingly, the monthly average concentration levels of PM10 and PM2.5 are similar, up to 30 µg/m³, in both time periods 2000-2001 with exception of the winter months, and almost independent of the TSP concentrations. This means, that the concentration levels of small particles (< 10 µm) are clearly affected by long-range transport, while TSP concentrations are mostly influenced from nearby sources under certain meteorological conditions.

The annual average concentration for PM10 (40 µg/ m³, 2001), was found to be almost similar to the value given by Zappoli et al. [4] for Northern Italy (38 µg/ m³), Lenschow et al. [5] for the background sites of the city of Berlin (ca. 25 µg/ m³) and much higher as compared to the concentration measured in a background site in Sweden of ca. 6 µg/ m³ [4].
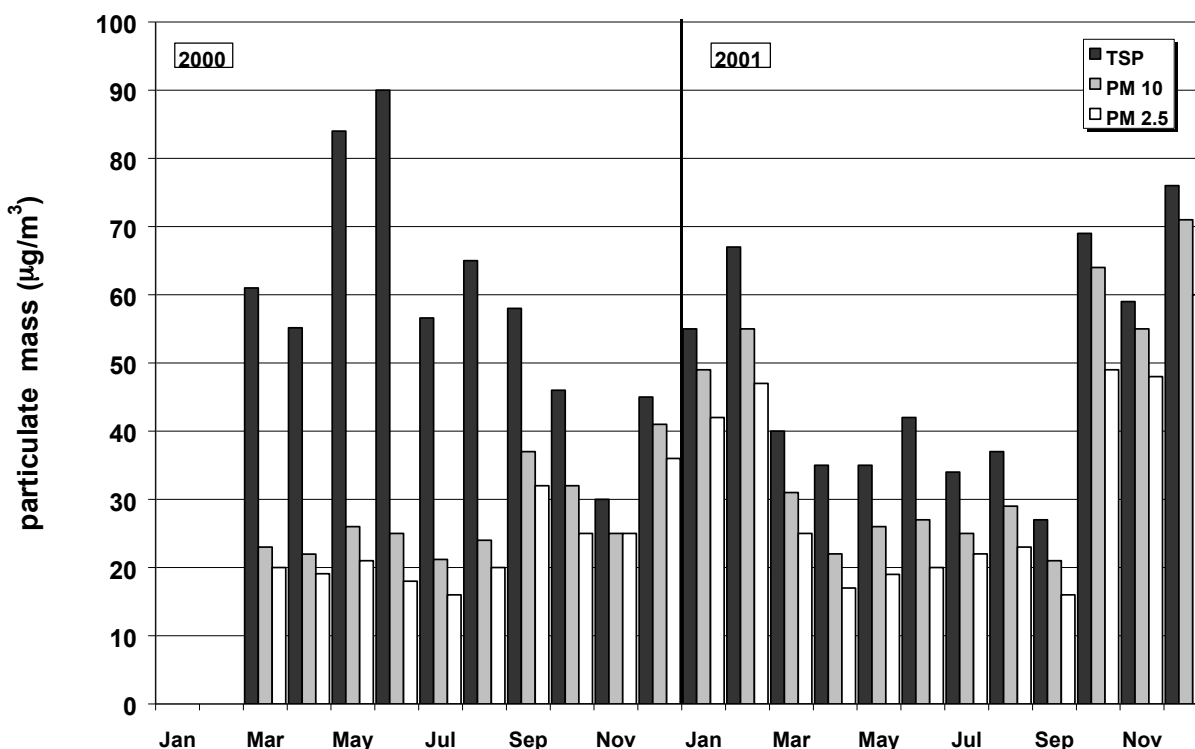
FIGURE 1 - The monthly average concentrations of TSP, PM10 and PM2.5, calculated from 24h daily collected samples, show greater annual changes for TSP during the measurement period than for PM10 and PM 2.5.
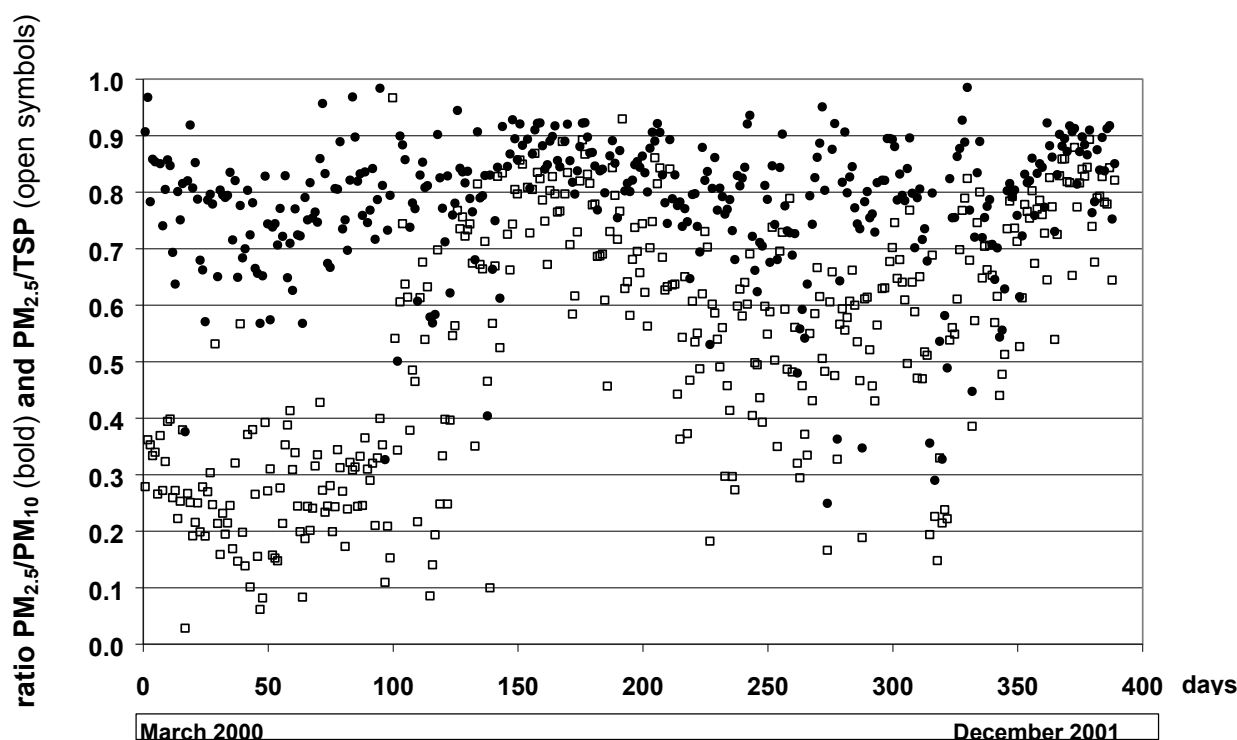
FIGURE 2
The ratios PM2.5/PM10 and PM2.5/TSP have been calculated on the basis of daily, simultaneously collected 24h samples. The ratio PM2.5/TSP (open symbols) shows annual changes with a maximum contribution of PM 2.5 to the total particulate mass during spring/summer and in late December, while the ratio between PM2.5/PM10 (closed symbols) does not show significant annual difference.

Figure 2 shows the ratio of PM2.5 to PM10 and TSP calculated from the daily 24h values. PM2.5 and PM10, respectively, may contribute 40-90% to the total particle mass. The PM2.5/TSP ratio varies between 0.20-0.89 with a mean value of 0.53, which is slightly lower than the ratio PM10/TSP with 0.60 in our as well as the ratio PM10/TSP calculated in other studies [5]. The ratio PM2.5/PM10 varies from 0.76 to 0.96 for the period March-December 2000 and from 0.73 to 0.88 for the period January-August 2001, which results in a mean ratio over the investigated period of 0.78. Hence, the main part of the PM10 is composed by particles with a diameter less than 2.5 μm. Since very few data on PM2.5 measurements are available until now, a conversion factor of 0.7/0.8 for the calculation of PM2.5 concentrations from PM 10 data can be used as a first approximation.

In view of possible health effects from the exposure to fine particles, there is a need for additional measurements to obtain reliable data for use in epidemiological studies. For an efficient implementation of the new air quality policy of the EU, it seems further necessary to focus on source apportionment studies for fine particles and to quantify the part, which may be attributable to long-range transport of these pollutants. In addition, the occurrence of particle-associated organic compounds of known toxicological relevance on ultra-fine particles will be a challenging task for future investigations, since high accumulations of such toxic chemicals were found onto particles with diameters less than 1 μm [6].

ACKNOWLEDGEMENT

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FORMATION OF DIOXINS FROM NEWSPAPER COMBUSTION IN THE PRESENCE OF SODIUM CHLORIDE AND POLYVinylidene CHLORIDE

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SUMMARY

Exhaust gases were collected from the combustion of newspapers alone, but also from newspapers impregnated with sodium chloride (NaCl), and mixed with polyvinylidene chloride (PVDC). The samples were analyzed for dioxins by high-resolution gas chromatography/ mass spectrometry. The total amounts of dioxins found in the samples were 21.6 pg g^{-1} from newspapers alone, 51.9 pg g^{-1} from newspapers with NaCl, and 1525 pg g^{-1} from newspapers with PVDC. It is postulated that there is a correlation between dioxin formation and chloride content. Formation of total PCDFs was considerably higher than that of total PCDDs, except in the case of newspapers alone. The total PCDF/total PCDD ranged from 0.16 (newspaper alone) to 16.4 (newspaper with PVDC). These results indicate that NaCl and PVDC contribute significantly to dioxin formation from waste materials combusted in incinerators.

KEYWORDS: Dioxins, combustion, newspaper, sodium chloride, polyvinylidene chloride.

INTRODUCTION

Incineration is widely used in the treatment of municipal solid waste. Incineration of wastes is expected to further increase in the future due to its two significant advantages over other methods of waste disposal: mass/ volume reduction and energy recovery. However, the major disadvantage of the incineration process is the emissions of toxic air pollutants, including polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (PCDD/Fs), etc. [1]. These toxic organic compounds could be released from the combustion of waste materials [2–4].

One of the main pathways has been proposed so far to explain the formation of PCDD/Fs during incineration: pyrosynthesis, that is, high temperature processes [5]. For example, dioxins are formed from natural woods and waste woods by combustion [6, 7]. They are also formed from sodium chloride (NaCl)-impregnated woods [6] and a mixture of wood and plastic wastes [8] during combustion. There have been many studies on the formation of dioxins under various conditions [9–12].

The source of chloride has been one of the major concerns in studies of dioxin formation in furnaces. It is found that both organic and inorganic chlorides can be a precursor of dioxins by the combustion of waste materials. There are two hypotheses about the role of chloride contents in dioxin formation upon combustion. One is that the content of chloride plays an important role in dioxin formation [13,14]. Another is that dioxin formation during combustion is not dependent on the content of chloride [15]. However, there are still many unknown formation mechanisms of dioxins in the high temperature processes.

In other study, the information on temperature dependence for the formation of dioxins has not been obtained, because the reaction temperature in the incinerator was obscurely controlled [16]. Therefore, in the present study exhaust gas was collected at the outlet of a tube-type furnace, in which newspapers alone, and newspapers mixed with NaCl or PVDC were combusted at 500 °C. Samples collected were analyzed for dioxins by gas chromatography/mass spectrometry (GC/MS) in order to investigate the characterization of dioxin formation during the combustion of the waste materials.

MATERIALS AND METHODS

Newspapers (top circulation in Japan) purchased from a local store were soaked in a 3% NaCl solution for 1h. The newspapers were dried in an electric oven at 120 °C prior to use in the experiment. A PVDC sheet (saran rap) was bought from Asahi Kasei Co., Ltd. (Tokyo, Japan).

Isotope-labeled dioxins for internal standards (1000 ng mL^{-1}) were obtained from Wellington Laboratories, Inc. For the solution of cleanup-spike recovery test, a 2-μL standard solution containing 1 pg μL^{-1} each of [^{13}C]-2,3,7,8-TCDD, 1,2,3,7,8-PCDD, 1,2,3,4,7,8-HxCDD, 1,2,3,6,7,8-HxCDD, 1,2,3,7,8,9-HxCDD, 1,2,3,4,6,7,8-HpCDD, [^{13}C]-2,3,7,8-TCDF, 1,2,3,7,8-PCDF, 2,3,4,7,8-PCDF, 1,2,3,4,7,8-HxCDF, 1,2,3,6,7,8-HxCDF, 1,2,3,7,8,9-HxCDF, 2,3,4,6,7,8-HxCDF, 1,2,3,4,6,7,8-HpCDF, 1,2,3,4,7,8,9-HpCDF, and 2 pg μL^{-1} each of [^{13}C]-OCDD and OCDF was prepared. For the solution of the internal standard, a 2-μL standard solution containing 1 pg μL^{-1} of [^{13}C]-1,2,3,4-TCDD was prepared.

All organic solvents for dioxin analysis were purchased from Wako Pure Chemicals Co., Inc. (Tokyo, Japan).

The combustion tests were performed in a tube-type furnace (Fig. 1). The furnace consisted of a quartz tube of 70 cm length and 3 cm inner diameter as well as an enclosing furnace of 32 cm length. A thermocouple was used to detect the temperature of the combustion zone. At the beginning of a test, the tube furnace was first heated up to 500 °C, then air was introduced in front of the quartz tube at 500 mL min^{-1}. After the furnace had reached 500 °C, sample was pushed into the combustion zone of the furnace. Three different samples were combusted in the furnace, that is, Sample I: 10 g of newspapers, Sample II: 10 g of NaCl-impregnated newspapers, and Sample III: 10 g of newspapers mixed with 0.3 g of PVDC. All samples were combusted at 2 h, and then exhaust gas samples were collected for 4 h at room temperature.

Dust in the exhaust gas was collected on silica wool. The exhaust gas was next drawn into two 1-L impingers connected in series. The two impingers contained 250 mL of toluene. The second impinger was further connected to a column packed with 5 g of XAD-2 resin (surface are: 304 m^2 g^{-1}), which was interfaced to a 1-L impinger containing 50 mL toluene. The exhaust gas was drawn using a diaphragm vacuum pump at a flow rate of 500 mL min^{-1}.

After the furnace was cooled to the room temperature, silica wool, XAD-2 resin and residues of samples were extracted for 24 h with toluene using Soxhlet extractor. The quartz tube and connection lines were washed with 100 mL toluene. The extracts and washes were then combined. Dioxins standards were added here for cleanup recovery tests. The combined samples were concentrated by a rotary evaporator, and were cleaned with multilayer silica gel column chromatography. The sample was further cleaned with a 120-mL hexane/dichloromethane (1/1) solution using alumina column chromatography. After 2 pg of ^{13}C-labeled 1,2,3,4-TCDD was added to each sample as internal standard for quantitative analysis of dioxins, the volume of sample solutions was adjusted to exactly 50 μL under nitrogen flow.

The PCDD/Fs analysis was performed using a Hewlett-Packard (HP) model 6890 GC interfaced with a JMS-700 double focus MS (JEOL, Japan). The GC was equipped with a splitless injection system and a CP-Sil 88 capillary column (60 m × 0.25 mm i.d.) for Cl$_{4–6}$ dioxins or a CP-Sil 8 capillary column (30 m × 0.25 mm i.d.) for Cl$_{7,8}$ dioxins. The oven temperatures were programmed to hold 100°C for initial 1 min, to increase from 100 to 180°C at a rate of 10°C min^{-1} and from 180 to 230°C at a rate of 3°C min^{-1} and to hold at 230°C for 45 min for the
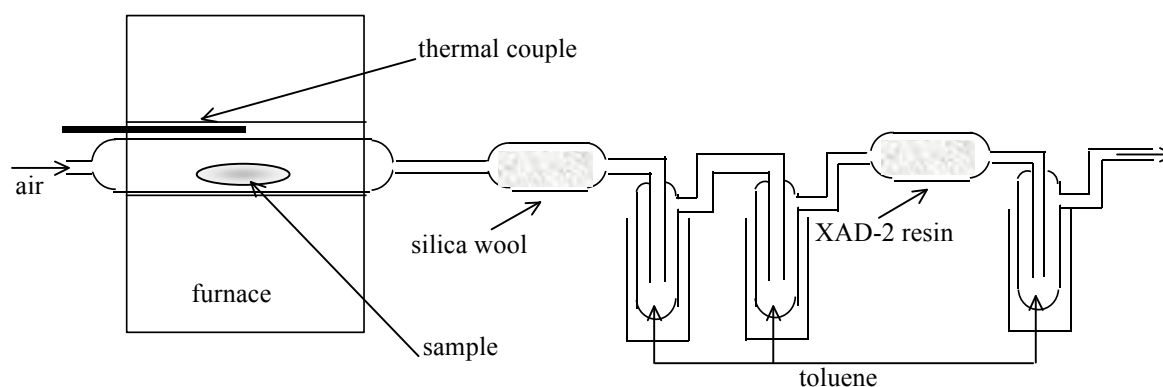
FIGURE 1 - Schematic of the combustion and collection system.

TABLE 1 - Amount of dioxins produced by combustion of newspapers.

4CDF	newspaper (pg/g)	newspaper+NaCl (pg/g)	newspaper+PVDC (pg/g)	4CDD	newspaper (pg/g)	newspaper+NaCl (pg/g)	newspaper+PVDC (pg/g)
1368	n.d.	1.10	48.2	1368	n.d.	n.d.	n.d.
1468	n.d.	1.92	102	1379	n.d.	n.d.	n.d.
2468	0.32	0.46	62.9	1369	n.d.	n.d.	n.d.
1247/1347/1378/1346/1248	1.29	0.82	29.3	1247/1248/1378/1468	n.d.	n.d.	n.d.
1367/1348/1379/1246	n.d.	n.d.	25.3	1246/1249	n.d.	n.d.	n.d.
1268/1467/1478	0.12	0.99	n.d.	1268	n.d.	n.d.	n.d.
1369/1237	0.15	2.46	n.d.	1478	n.d.	n.d.	n.d.
2368	n.d.	n.d.	n.d.	1279	n.d.	n.d.	n.d.
2467/1238/1236/1469/1678/1234	n.d.	3.03	391	1234/1236/1289	n.d.	n.d.	n.d.
1278	n.d.	n.d.	n.d.	1237/1238	3.02	n.d.	n.d.
1349/1267	0.21	n.d.	n.d.	2378	n.d.	n.d.	n.d.
2378/2348/2347/2346/1249/1279	0.74	8.88	393	1239	1.50	n.d.	n.d.
2367	n.d.	n.d.	40.7	1278	0.66	n.d.	n.d.
3467/1269	n.d.	n.d.	38.9	1267	1.14	n.d.	n.d.
1239	n.d.	n.d.	n.d.	1289	3.67	18.8	20.8
1289	n.d.	n.d.	65.9	Total	9.98	18.8	20.8
Total	2.83	19.7	1197				
5CDF				**5CDD**			
13468/12468	n.d.	0.57	35.7	12468/12479	n.d.	n.d.	n.d.
13678	n.d.	0.19	n.d.	12469	n.d.	n.d.	n.d.
12368/12478/13467/13478/12467	0.06	0.97	15.7	12368	n.d.	n.d.	n.d.
13479/14678	n.d.	n.d.	19.5	12478	n.d.	n.d.	n.d.
12479/13469	n.d.	n.d.	n.d.	12379	n.d.	n.d.	n.d.
23468/12469/12347/12346	n.d.	0.35	n.d.	12369	n.d.	n.d.	n.d.
12348	n.d.	n.d.	n.d.	12467/12489	n.d.	n.d.	n.d.
12378	n.d.	1.05	17.8	12347	n.d.	n.d.	n.d.
12367	n.d.	0.60	56.4	12346	n.d.	n.d.	n.d.
12678/12379	n.d.	0.19	5.20	12378	n.d.	n.d.	n.d.
23478/12489/12679/12369	0.08	0.67	4.70	12367	n.d.	n.d.	n.d.
23467	n.d.	n.d.	29.1	12389	n.d.	n.d.	n.d.
12349	n.d.	n.d.	5.86	Total			
12389	n.d.	n.d.	12.5				
Total	0.14	4.59	202				
6CDF				**6CDD**			
123468	n.d.	n.d.	n.d.	124679/124689	n.d.	n.d.	n.d.
134678/124678	n.d.	n.d.	n.d.	123468	n.d.	n.d.	n.d.
134679	n.d.	n.d.	n.d.	123679/123689	n.d.	n.d.	n.d.
124679	n.d.	n.d.	n.d.	123469	n.d.	n.d.	n.d.
124689	n.d.	n.d.	14.2	123478	n.d.	n.d.	n.d.
123467/123478	n.d.	0.40	6.88	123678	n.d.	n.d.	n.d.
123678	n.d.	n.d.	7.88	123467/123789	n.d.	n.d.	n.d.
123479	n.d.	n.d.	n.d.	Total			
123469/123679	n.d.	n.d.	n.d.				
123689	n.d.	n.d.	n.d.				
234678	n.d.	0.43	n.d.				
123789	n.d.	n.d.	5.21				

TABLE 1 (*Continued*)

123489	n.d.	n.d.	n.d.				
Total		0.83	34.2				
7CDF				**7CDD**			
1234678	n.d.	n.d.	11.5	1234678	1.37	0.94	19.5
1234689	n.d.	n.d.	3.47	1234679	1.55	1.72	20.0
1234679	n.d.	n.d.	2.57	Total	2.92	2.66	39.5
1234789	n.d.	n.d.	3.71				
Total			21.3				
8CDF				**8CDD**			
	n.d.	2.30	2.39		5.70	3.05	7.07

n.d. = not detetcted

CP-Sil 88 column, and programmed to hold 100°C for initial 1 min, to increase from 100 to 200°C at a rate of 10°C min^{-1} and from 200 to 280°C at a rate of 3°C min^{-1} and to hold at 280°C for 15 min for the CP-Sil 8 column. Helium was used as a carrier gas with a flow rate of 1 mL min^{-1}. The injector temperatures were 230°C for the CP-Sil 88 column and 280°C for the CP-Sil 8 column. MS ion source temperatures were 230°C for the CP-Sil 88 column and 280°C for the CP-Sil 8 column. MS ionization voltage was 38 eV. Detection limit for 2,3,7,8-TCDD was 2 fg in this study.

RESULTS AND DISCUSSION

Table 1 shows the results of dioxin analyses of the exhaust gas samples obtained from the incinerator. The amounts of dioxin isomers formed in the Sample I, II and III were 0.06–3.67, 0.19–18.8 and 2.39–393 pg g^{-1}, respectively. Figure 2 shows the total amounts of PCDD/Fs formed by the combustion of newspapers at 500°C. When newspapers alone were combusted, the total amount of PCDD/Fs formed was 21.6 pg g^{-1} (total PCDF/total PCDD was 0.16). This value is quite low as compared with those of samples with chloride. These results are similar to those reported in the combustion of woods [7, 8]. When newspapers were impregnated with 3 % NaCl as chlorine source, amount of dioxin formed increased *ca.* 2.5-fold, suggesting that NaCl is a possible chloride source for dioxin formation. It was reported that some food products containing NaCl produced dioxins upon combustion [17]. Furthermore, when newspapers were combusted with PVDC, 1525 pg g^{-1} of dioxin was formed. In particular, the PCDF generated 500 times higher than newspaper alone. It was reported that materials with a higher percentage of chloride content produced more dioxins and that there is clear correlation between dioxin generation and chloride content [18]. In this study, newspapers with inorganic chloride source (NaCl) or organic chloride source (PVDC) produced significantly higher amounts of dioxins as compared with newspapers alone.

Formation of total PCDFs was considerably higher than that of total PCDDs in the Samples II and III (Fig. 2). For example, total PCDFs was 16.4-fold total PCDDs in Sample III. The total PCDF/total PCDD ranged from 0.16 (Sample I) to 16.4 (Sample III). These results were consistent with the results obtained from municipal solid waste combustion [10].
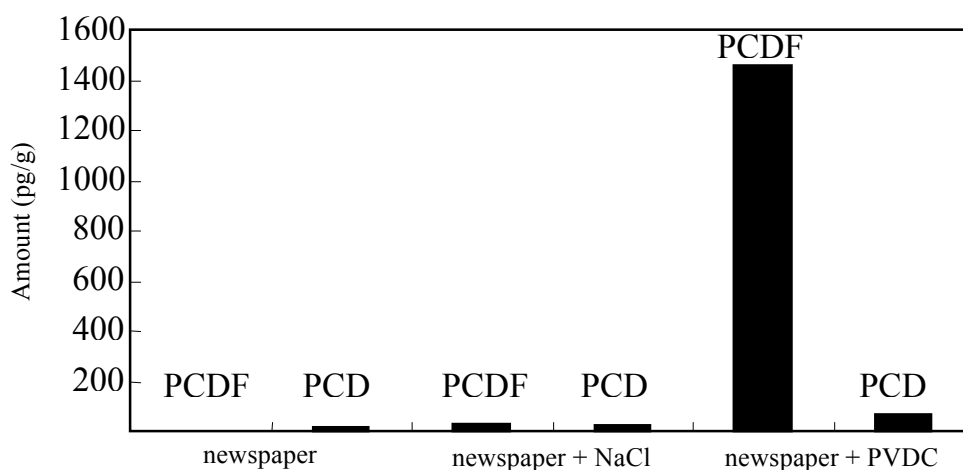

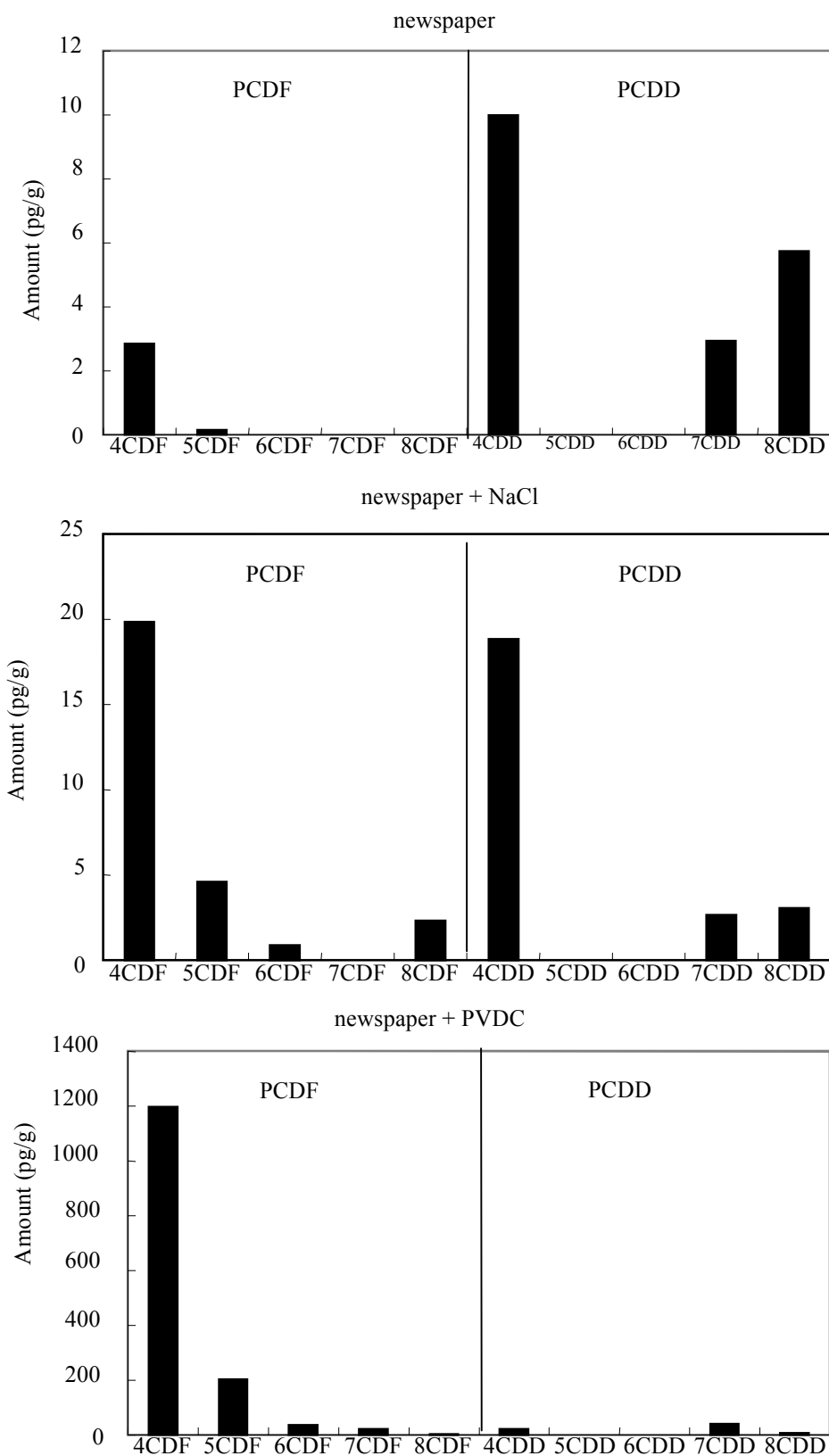**FIGURE 2 - Total amounts of PCDD/Fs produced by the combustion of newspapers at 500 °C.**

FIGURE 3
Amounts of PCDD/F isomers produced by the combustion of newspapers at 500 °C.

The amount of PCDD/F isomers produced by combustion of newspaper is shown in Fig. 3. When newspaper alone was incinerated, predominantly TCDD was generated. On the contrary, when newspaper was combusted in the presence of PVDC, predominantly TCDF was generated and the concentration of PCDF isomers was decreased with increasing the concentration of chloride. In the case of newspaper, in the presence of sodium chloride TCDF concentration was almost the same as TCDD.

In the present study, both organic and inorganic chlorides were shown to relate to dioxin formation as a source of chloride. There are many reports on dioxin formations from the combustion of various waste materials with organic chloride. In particular, polyvinyl chloride (PVC) produced dioxins in high amounts via combustion or thermal degradation [19]. It was reported that chloride sources in municipal wastes are 50% from NaCl and 45% from PVC [9]. However, there have been only a few reports on the possible formation of dioxins from the reaction of inorganic chloride, such as NaCl and HCl, with waste materials during combustion. When HCl was introduced into gasoil combustion, gases including methane, propane and ethylene generation of dioxins was observed [20]. It was postulated that HCl is formed at first from NaCl or PVC by high temperature and that dioxins are subsequently produced [21]. Dioxins were formed under electrostatic precipitation conditions in the presence of HCl and/or $CuCl_2$ [22]. Organic chloride (tetrachloroethylene) with a catalyst such as iron(III), tin(II) and copper(II) promoted the formation of particle-bound dioxins in combustion experiments. On the other hand, inorganic chloride (NaCl) promoted the formation of dioxins more effectively in the gas phase than in the particle phase [23].

CONCLUSION

Exhaust gas from the combustion of newspaper alone and newspapers mixed with NaCl or PVDC were analyzed for dioxins. Total amounts of dioxins found in the samples were 21.6 pg g^{-1} from newspapers alone, 51.9 pg g^{-1} from newspapers impregnated with NaCl, and 1525 pg g^{-1} from newspapers with PVDC. When newspaper alone was incinerated, predominantly TCDDs were generated. On the contrary, when newspaper was combusted in the presence of PVDC, predominantly TCDFs were generated and the concentration of PCDF isomers was decreased with increasing the concentration of chloride. These results indicate that NaCl and PVDC contribute significantly to dioxin formation from the waste materials combusted in incinerators.

ACKNOWLEDGMENTS

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FEB

CHARACTERISTICS OF SURFACE OZONE CONCENTRATIONS IN URBAN ATMOSPHERE OF ISTANBUL: A CASE STUDY

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SUMMARY

History of photochemical air pollution in Istanbul is not long. In recent years, the number of motor vehicles in Istanbul increased at a very fast rate. As a consequence, air quality problems in this city shifted from conventional pollutants to the secondary pollutants such as O_3. In this study we present the recent both chemical and meteorological data for a typical summer month in Istanbul. The purpose of this analysis is to examine the variations in ozone-conducive meteorological conditions in the urban atmosphere of Istanbul.

KEYWORDS:
Surface ozone, episode, urban meteorological conditions.

INTRODUCTION

Generally, urban areas are impacted mostly by traffic emissions in the summer season. Ozone is a secondary pollutant produced from a variety of natural and anthropogenic precursors, that include industrial and vehicular emissions of volatile organic compounds (VOCs) and nitrogen oxides (NO_x). They are formed in the atmosphere through a serious of photochemical reactions from natural and anthropogenic precursors in the presence of strong solar radiation. A well-known general reaction mechanism for ozone at the surface boundary layer involves reacting NO_x and NMHC with solar radiation [1, 2].

Recently, ozone chemistry and the related meteorology have been extensively studied around the world. However, the history of photochemical air pollution in Istanbul, Turkey, is not long. Topcu and Incecik [3] explained the preliminary results of the ozone measurements in the urban atmosphere of Istanbul. Even in Istanbul, where high ozone levels now occur frequently, O_3 pollution was low in 1998 and 1999. The city of Istanbul did have serious SO_2 and TSP pollution up to mid 90's [4]. The switching of fuel from high sulfur oil and coal briquette to clean heat sources (low-sulfur oil and liquefied natural gas) caused the disappearance of classical air pollution in the city. In recent years, the number of motor vehicles increased at a very fast rate in Istanbul and as a consequence, the air quality problems in the city shifted from those caused by primary pollutants to the secondary pollutants such as O_3. Photochemical O_3 problems became an important issue during the hot summer of 2001 in Istanbul. The photochemical ozone pollution is promoted by increasing solar radiation, increasing temperature dependent emissions and reducing ventilation of the source region. It is assumed, however, that these characteristics were dependent on the combined effects of precursor emissions of ozone and meteorological conditions such as synoptic pressure systems, solar radiation, temperature, cloud cover and wind speed and direction [2, 5, 6]. Furthermore, a discussion of the influence of key factors on high ozone concentrations has been given.

In this study, we present the processes influencing the surface ozone pollution in July 2001 by a data set selected from the measured period as a case study. As a result, the study will qualitatively identify the meteorological features present during high ozone days in July 2001.

GENERAL CHARACTERISTICS OF THE ENVIRONMENT IN ISTANBUL

The population of Istanbul currently stands at about 10.0 million. The main built-up areas and urban centers are situated on both sides of the city. There are about 1.5 million motor vehicles registered in the city. The Bosphorus is located along a southwest-northeast axis in Istanbul has about 1.5 km width strait between the two parts of the city. There are two bridges on the Bosphorus (Bosphorus and Fatih Sultan Mehmet bridges). It is reported that around 60 million vehicles passed on both the bridges in 2001.

Istanbul (41^oN; 29^oE) lies between different climatic bands. It is also common for different climatological regions to dominate along the Sea of Marmara, the Black

Sea and through the Bosphorus throughout the year. The daily mean temperature is about 21.8°C in summer (June-August) and is about 6.5°C during winter (December-February). In summer, a high-pressure system covers the eastern Mediterranean and Balkan area up to the Black Sea and a thermal low appears over the Asia Minor [4, 7–10]. As a result, cloudless skies are more frequent during summer months in the city. The predominant winds blow N/NE and SSW/SW in the city.

Furthermore, sea breeze circulations are often observed under light synoptic winds in the summer months.

MONITORING SITES AND DATA

In Istanbul, the hourly ozone concentrations have been measured and monitored since 1998 by the Istanbul Municipality at 2 selected stations (Kadikoy and Sarachane), which are located at Asian and European side of the city respectively. Both stations are close to heavily used commuter traffic in the city. In this study, we used ozone data obtained from the Kadikoy station only. Due to the missing values of the ozone data at Sarachane, ozone data in European side of the city could not be used for July 2001. In particular, the Kadikoy monitoring site is likely to be strongly influenced by the proximity to the major transportation routes in the city. The two major transportation routes (E5 and O2) connecting both bridges are situated at 3 and 8 km, respectively, north of Kadikoy ozone monitoring station in the Asian side.

The measurement system of ozone is $O_3 41M$ of Environment S.A [11]. The system is based on the absorption of UV radiation. In this study, unfortunately, it was not possible to obtain all the necessary chemical data to explain the characteristics of the peak levels. The ambient data on NO_x, and hydrocarbon were not available at Kadikoy.

The ozone precursor data set as well as NO and NO_2 were obtained at Sarachane. The NO_x analyzer (AC31M of Environment S.A) was located only in the Sarachane air pollution monitoring station [11]. The measurement system is based on the chemiluminescense effect produced by the oxidation of NO by O_3 molecules. In addition, the hourly wind and temperature, and cloud cover data measured at the Goztepe Meteorological Station, in Istanbul Asian side at 07, 14 and 21 hours, were used to construct the typical meteorological characteristics of high O_3 days. This station is nearby Kadikoy air quality monitoring station.

RESULTS AND DISCUSSION

The ozone season is usually defined as a period from 1 April to 30 September. In this study, we considered a period from 1 May to 31 July 2001 at the Kadikoy station (The ozone concentrations could not be measured at the Kadikoy monitoring station in April, August and September 2001). Fig.1 shows a time series of daily peak ozone concentrations in this period at Kadikoy.

The diurnal variation of ozone in the surface air is a well-known phenomenon. The average diurnal variation of ozone and its standard deviation throughout the month of July 2001 is shown in Fig. 2. The time of the maximum ozone concentration is from 15:00 to 17:00 h, almost with 70% 2-4 h after noon. 30% of the maximum ozone concentration has occurred in the late afternoon. While the daytime increase in ozone is attributed to photochemical production, the late afternoon maximum is totally inconsistent with local ozone production. This can be explained that it is more consistent with the measured ozone having been produced earlier in the day, somewhere with higher solar irradiance, and transported to the site.
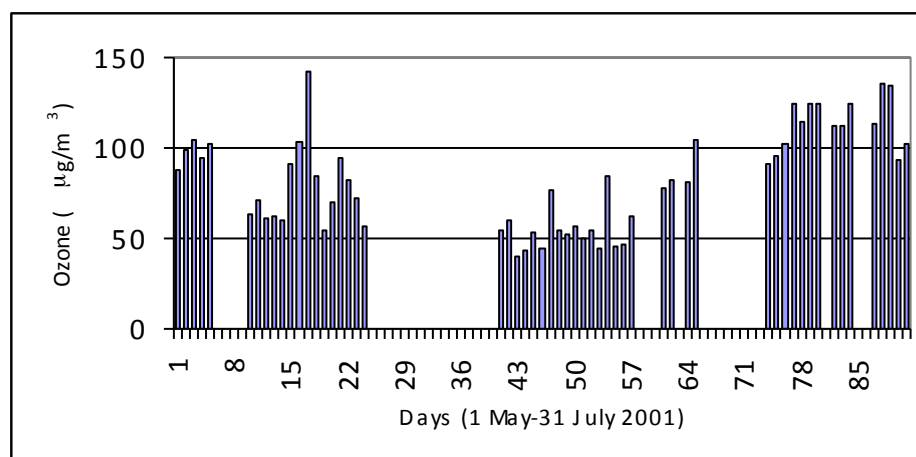
FIGURE 1
Time series of daily maximum 1h O_3 from 1 May to 31 July 2001 in Kadikoy.
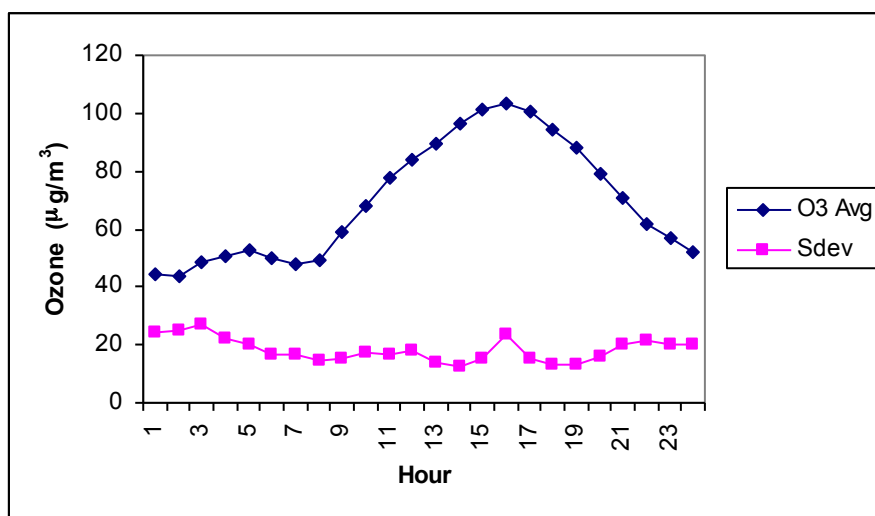
FIGURE 2
The average diurnal variation of ozone throughout the month of July 2001.

The average NO_x concentration in July 2001 was $67\mu g/m$ [3, 11]. The diurnal variation in NO_x had particularly a morning peak, an afternoon minimum and the nighttime maxima. The peaks in traffic emissions in the morning and evening were clearly discernible at the Sarachane Station. During the morning hours, lower boundary layer heights reduced the mixing processes. The low levels of ozone during the early morning hours were due to the combined effects of chemical loss by NO and NO_2 species and the suppressed boundary layer mixing processes. NO concentrations at Sarachane were generally found to be above the threshold levels for ozone concentrations. In July 2001, the Kadikoy station showed a bimodal diurnal pattern in O_3 characterized by a primary peak in mid afternoon corresponding to maximum solar radiation, and a secondary lower peak in the early morning. The lowering of the boundary layer overnight and diminished wind speeds may lead to these early morning peaks in the city. On the other hand, higher wind speeds may lead to a decrease of ozone concentrations in the summer season. When the wind speed is higher than 5 m/s, a decreasing trend in ozone occurs and this is due to increased dilution of ozone formed within the atmospheric boundary layer.

As found in numerous studies, certain atmospheric conditions, such as solar radiation, high temperatures, weak winds and well-defined boundary layers are required for the formation and accumulation of high ozone concentrations [2, 5, 6]. These conditions promote photochemical ozone pollution by increasing solar radiation, increasing temperature-dependent emissions and reducing the ventilation of the source region. It is assumed, however, that these characteristics are dependent on the combined effects of precursor emissions of ozone and meteorological conditions such as synoptic pressure systems, solar radiation, temperature, cloud cover and wind speed and direction.

In this study the influence of key factors on high ozone concentrations is discussed. The wind rose and maximum temperatures show that high ozone levels are usually associated with light southwesterly winds in the morning and maximum daytime temperatures in excess of 30 °C. Richardson number is one of the parameters in understanding the combined effects of both thermal and dynamical instabilities of air within the lower atmosphere. Furthermore, we used Ri, a non-dimensional stability parameter, which is defined here as the vertical gradient of temperatures divided by the vertical gradient of horizontal wind component [12]. Accompanying large-scale motion, the meteorological conditions in summer are unstable. The ozone measurements were obtained for only 20 days in July in Kadikoy. The average daily maximum ozone for the 20 days is $108\mu g/m^3$. This result brings our attention to Kadikoy ozone data set for July 2001. In order to analyze the characteristics of high ozone days, we defined the high ozone day as when a site records an excess of $100\mu g/m^3$. A total of 58 hrs with concentrations above 1-hr/$100\mu g/m^3$ in July 2001 with clear weather conditions in the city was examined on high ozone days. The thirteen events studied and the associated meteorological conditions are summarized in Table 1. Ri numbers for 0000 UTC (Universal Time Coordinated) and 1200 UTC are added to the table. Maximum solar radiation ranged between 771-913 W/m^2. The daily maximum air temperatures on the high ozone days were recorded generally above 30 °C, with clear weather conditions.

The morning and afternoon mean wind speed and directions on the high ozone days are shown in Table 1. As can be seen from this table, most of the mean morning

wind speeds for the high ozone days are less than 2.0 m/s. The only two strong NNE morning winds are associated with the synoptic scale pressure systems occurring in the region. On the days, July 22 and 31, Istanbul and the surrounding area became cloudy in the early morning hours, which decreased throughout the day. The mean cloud cover is 5/10 for both days. The strong north winds may promote the transport of ozone, or precursors from major transportation routes (E5 and O2) situated in about 3-8 km north of the monitoring site. There is a large differ-

ence in the direction of mean wind speeds for high and non-high ozone hours. The wind roses shown in Fig.3 indicates that, the dominant flow directions of the high ozone hours in July 2001 are WSW with a percentage of 22.0 and NNE with 24%. There are also differences in the frequency of the calm winds (u ≤ 1m/s) between high and non-high ozone days. Calms are 15% or more of the total for high ozone hours and less than 11% for non-high ozone hours.

TABLE 1 - Daily peak ozone concentrations, meteorological variables and Ri numbers for high ozone days (O$_3$ >100µg/m^3) in July 2001.

Date	Daily Peak 1-hr O$_3$ µg/m^3	Mean WD 0700	Mean WD. 1400	Mean WSP 0700 m/s	Mean WSP 1400 m/s	Max. Temp °C	Max. SR W/m^2	Mean cloud cover 1/10	Ri 0000 UTC	Ri 1200 UTC
July 05	105	WSW	WSW	0.7	2.4	26.3	913	1	+129.0	Na
July 16	102	W	NNW	1.3	4.3	31.7	906	2	+0.8	-0.6
July 17	125	SW	WSW	0.9	2.4	30.0	771	3	+8.5	+0.0
July 18	115	W	NNE	1.4	5.6	30.1	865	1	+40.1	-0.2
July 19	125	WSW	NNE	1.2	4.2	32.1	906	0	Na	Na
July 20	125	W	NNE	1.0	4.7	33.2	913	0	+2.0	-3.2
July 22	112	NNE	NNW	3.9	3.3	28.9	891	5	+1.1	-24.2
July 23	112	WNW	ENE	1.9	3.9	31.3	907	0	+479.0	-10.1
July 24	125	SW	NNE	0.6	4.4	31.2	903	0	+3.2	-0.2
July 27	113	ENE	NE	1.6	4.3	32.3	894	0	+1.1	-7.5
July 28	136	SSW	NE	0.6	5.0	31.2	866	0	+1.9	-1.4
July 29	135	W	NNE	1.2	5.0	33.2	880	0	+355.0	-4.2
July 31	102	NNE	NE	4.9	5.5	31.2	889	5	+10.0	-11.0
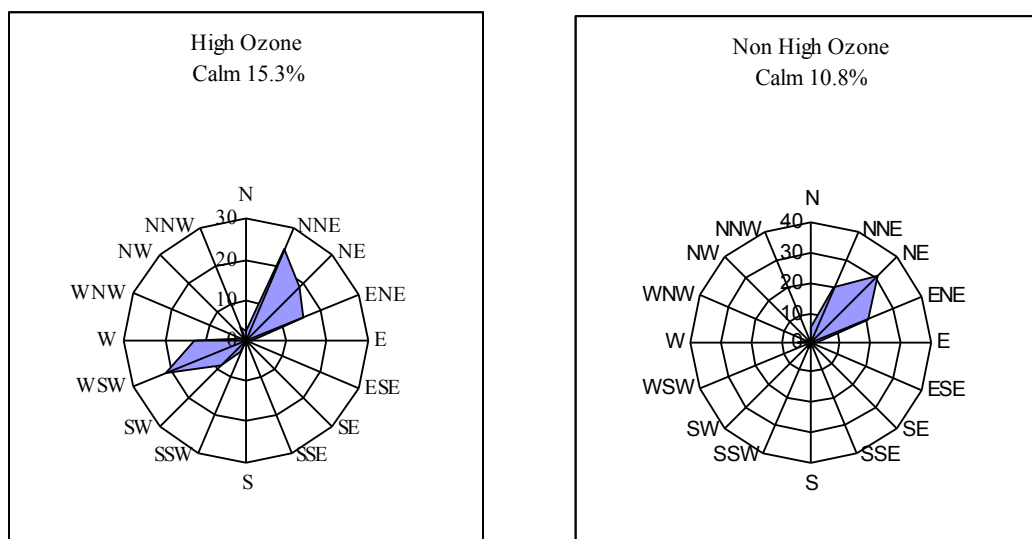
FIGURE 3
Wind roses for high ozone and non-high ozone hours in July 2001.

SUMMARY AND CONCLUSIONS

In this study, the surface O_3 pattern in a typical summer month of Istanbul is analyzed through interaction with its precursors and the influence of the meteorological conditions. The conditions leading to the formation of high ozone concentrations in the urban atmosphere of Istanbul are generally given as following:

1. A total of 58 hrs with concentrations above 1hr/$100\mu g/m^3$ was recorded in Kadikoy over a 20/31-day period. Thus, it is implied that on some days important O_3 exceedances persisted more than an hour per day in Kadikoy.

2. Weak morning surface winds, early morning strong stable conditions and higher precursor concentrations in the morning hours, are a cause for higher ozone concentrations; these conditions are most favorable for O_3 formation. Additionally, stagnant anticyclonic conditions may favor O_3 formation; as well as calm conditions may lead to higher ozone hours.

3. Strong NNE morning winds are associated with synoptic scale pressure systems in the region. This may promote the transport of O_3, or precursors from major transportation routes situated at about 2-7 km north of the monitoring site in the Kadikoy urban area. Ozone conducive meteorological conditions exist under stagnant high-pressure systems for the selected cases.

4. Convective atmospheric conditions, well-defined boundary layer and increased instability with the emissions may cause the peak ozone concentrations.

ACKNOWLEDGMENT

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FEB

ADSORPTION KINETICS OF VICTORIA BLUE ONTO PERLITE

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SUMMARY

The effects of various experimental parameters such as initial dye concentration, temperature and pH on adsorption capacity of victoria blue on perlite have been investigated. The extent of dye removal increased, with the increase in initial concentration, pH and temperature of the dye and contact time. Adsorption measurements show that the process is fast and the adsorption data were modeled using the first order kinetic equation. The results indicate that perlite could be employed as a low cost alternative to commercial adsorbents in wastewater treatment for the removal of colour and dyes. The activation energy was calculated as 2.4 kJ/mol.

KEYWORDS:
Perlite, victoria blue, kinetics of adsorption, dye.

INTRODUCTION

Effluents from the dyeing and finishing processes in textile industry are known to contain colour, high amounts of surfactants, dissolved solids and possibly heavy metals such as Cr, Ni and Cu. The effluents from the dyestuff manufacturing and some similar industries are also often highly coloured with a large amount of suspended organic solids and hence important sources of water pollution. From an environmental point of view, the removal of synthetic dyes is of great concern, since some of them and their degradation products may be carcinogens and toxic and, consequently, their treatment cannot depend on biodegradation alone. Hence, decolourization of dye house effluent via the removal of dyes has become an important aspect of textile wastewater treatment [1].

The environmental issues surrounding the presence of colour in effluents is a continuing problem for dyestuff manufacturers, dyers, finishers and water companies, because increasingly stringent colour standards are being enforced by regulatory bodies to reduce the quality of colour in effluent and water courses. At present, the problem is considered to be solely aesthetic rather than eco-toxicological. Even so, the problem of colour in wastewater is common to many dye houses [1, 2].

Biological treatment processes are reported to be efficient in the removal of suspended solids and reduction of chemical oxygen demands, but are largely ineffective in removing colour from wastewater [3]. Hence, investigations have been conducted on physico-chemical methods of removing colour from textile eflluent. These studies include the use of coagulants, oxidising agents, ultra-filtration, electro-chemical and adsorption techniques [1]. The advantages and disadvantages of each technique have been extensively reviewed. Of these methods, adsorption has been found to be an efficient and economic process to remove dyes, pigments and other colourants and also to control the biochemical oxygen demand. Activated carbon, inorganic oxides, natural adsorbents (such as clays and clay minerals, cellulosic materials, chitin and chitosan) have been extensively used as adsorbents [1, 4, 5].

Activated carbon adsorption has been found to be an effective and widely employed means of water and wastewater treatment. Despite the high adsorption capacity of activated carbon in removing a wide range of dissolved organics from industrial effluents including toxic ones, it is not economically attractive in wastewater treatment. Morever, about 30% of the activated carbon used is lost during conventional thermal regeneration process, and the regenerated carbon has a lower adsorption capacity than the initial. Therefore, the finding alternative low cost materials is highly desirable [6].

Perlite is a glassy volcanic rock, commonly light gray, with a rhyolitic composition and 2 to 5% of bound water [7]. Expanded perlite come into competition from various other industrial minerals [8]. Commercially, the term perlite includes any volcanic glass that will expand or "pop" when heated quickly, forming a light-weight frothy material. The temperature at which expansion takes place ranges from 760 to 1100 ^{0}C; a volume increase of 10 to 20 times is common [7]. However, this versatile light-weight material with its low bulk density continues to grow in popularity even though it is, by no means, the cheapest material [8]. Along the Aegean coast, Turkey possesses about 70% (70.10^9 tons) of the world's known perlite reserves [9]. The uses of expanded perlite are multifarious and based primarily upon its physical and chemical properties. Because of its low thermal conductivity,

high adsorption capacitiy, low bulk density, and fire resistance, perlite aggregate plasters have many advantages over conventional plaster. Over half of the perlite produced goes into the construction industry, in particular as aggregate in insulation board, plaster, and concrete. In cryogenic (extremely low temperature) applications, perlite is used to insulate storage vessels for liquefied gas. Expanded perlite is used as a rooting medium and soil conditioner, and also as a carrier for herbicides, insecticides and chemical fertilizers. Accurately sized perlite is used as an aid in filtering water and other liquids, in food processing and pharmaceutical manufacture. As most perlites have a high silica content, usually higher than 70%, they are chemically inert in many environments and, hence, are excellent filter aids and fillers in various processes and materials. Miscellaneous uses of expanded perlite include fillers or extenders in paints, enamels, glazes, plastics, resins, and rubber as well as an abrasive catalyst in chemical reactions and agent in mixtures for oil well cementing [10].

In our previous works, we investigated the electrokinetic properties [11] and surface titrations of perlite suspensions [12], and also the adsorption of victoria blue [4] and copper (II) [5] from aqueous solutions onto perlite samples. The present study is aimed to determine a convenient and economical method for victoria blue removal from water, e.g. using a low cost and an abundantly available adsorbent, and elucidate the adsorption kinetics and activation energy of the system.

MATERIALS AND METHODS

Materials

The perlite sample was obtained from "Cumaovası Perlite Processing Plants" of Etibank (İzmir, Turkey). The chemical composition of perlites found in Turkey is given in Table 1 [9].

TABLE 1 - Chemical composition of perlite.

Constituent	Percentage Present
SiO_2	71-75
Al_2O_3	12.5-18
Na_2O	2.9-4.0
K_2O	4.0-5.0
CaO	0.5-2.0
Fe_2O_3	0.1-1.5
MgO	0.03-0.5
TiO_2	0.03-0.2
MnO_2	0.0-0.1
SO_3	0.0-0.1
FeO	0.0-0.1
Ba	0.0-0.1
PbO	0.0-0.5
Cr	0.0-0.1

The perlite sample was treated before use as follows [4, 5, 11, 12, 14, 15]: the suspension containing 10 g L^{-1} perlite was mechanically stirred for 24 h, after waiting for about 2 min the supernatant suspension was filtered through a white-band filter paper $(\Phi$ 12.5 cm). The solid sample was dried at 110 ^{0}C for 24 h, then sieved (100 mesh sieve). Particles under 100 mesh are used in further experiments.

The cation exchange capacity (CEC) of perlite was determined by the ammonium acetate method (25.97 meg $100g^{-1}$), and density by the picnometer method (2.3 g cm^{-3}). The specific surface area of perlite was measured by BET N_2-adsorption (1.22 m^2 g^{-1}). All chemicals were of analytical grade.

Method

Victoria blue from Carlo Erba and was used without further purification, dried at 110 ^{0}C for 2 h befor use. All the victoria blue solutions were prepared with distilled water and used for adsorption kinetic studies onto perlite. In these systems, the dye concentration was 1.0 x 10^{-4} mol L^{-1}, except those in which the effect of the concentration was investigated. For the experiments of adsorption kinetics, 10 g-perlite samples were added to 1 L of victoria blue solution at a desired concentration, temperature and pH. The pH of the solution was adjusted with NaOH or HNO_3 solution by using an Orion 920A pH-meter with a combined pH electrode, standardized with NBS buffers before measurements. A preliminary experiment revealed that about 30 min is required for the adsorption process to reach the equilibrium concentration. Therefore, the mixture was continuously agitated by a magnetic stirrer at 30 ^{0}C and 500 rpm for 30 min. A constant temperature bath was used to keep the temperature constant. At the end of the adsorption period, the solution was centrifuged for 15 min at 5000 rpm. The amount of dye adsorbed on perlite at any time, t, was determined from absorbance measured with a Cary |1E| UV-Vis spectrophotometer (Varian) at 584 nm (maximum). The amount of dye adsorbed was calculated from the concentrations in solutions before and after adsorption process. Each experimental point was an average of two independent adsorption tests [16].

RESULTS AND DISCUSSION

Adsorption rate

The adsorption experiments were carried out at different experimental conditions and the results obtained are discussed below.

Effect of contact time and initial adsorbate concentration on adsorption process

The effects of contact time and initial concentration of dye on the extent of removal of victoria blue on perlite were investigated. Fig. 1 shows the extent of dye adsorption as a function of reaction time. The results obtained

show that dye adsorption reaches an equilibrium adsorption in 30 min. Based on these results, 30 min was taken as the equilibrium time in kinetic adsorption experiments. The curves in Fig. 1 represent the amounts of victoria blue adsorbed onto perlite from 1.0×10^{-4}, 1.5×10^{-4} and 2.0×10^{-4} mol L^{-1} solutions at pH 6 and 30 ^{0}C. Q_e is the ratio of amount of victoria blue adsorbed per g of adsorbent (mol g^{-1}). The amount adsorbed increased exponentially with the increase in initial concentration of victoria blue. This indicates that there exists reduction in immediate solute adsorption, owing to the lack of available active sites required for the high initial concentration of victoria blue. Similar results have been reported on the extent of removal of dyes [17, 18], metal ions [19], and carboxylic acids [20]. It is further noted that the amount of victoria blue adsorbed decreases from 1.1×10^{-5} to 7.4×10^{-6} mol g^{-1} by decreasing the concentration of the adsorbate solution from 2.0×10^{-4} to 1.0×10^{-4} mol L^{-1} at pH 6 at 30 ^{0}C.

Effect of temperature on adsorption process

The temperature has two major effects on the adsorption process. Increasing the temperature is known to increase the rate of diffusion of the adsorbate molecules across the external boundary layer and in the internal pores of the adsorbent particles, owing to the decrease in viscosity of the solution. In addition, changing the temperature will change the equilibrium capacity of the adsorbent for a particular adsorbate [21]. Fig. 2 shows the results of contact time experiments carried out at different temperatures for victoria blue adsorption on perlite. The

removal of victoria blue by adsorption on perlite increeases from 7.4×10^{-6} to 9.9×10^{-6} mol g^{-1} by increasing the temperature of the solution from 30 ^{0} to 60 ^{0}C, indicating the process to be endothermic. This kind of temperature dependence of the amount of the dye adsorbed may be correlated with penetration of dye into the perlite. In fact, a possible mechanism of interaction is the reaction between the hydroxyl endgroups of the perlite and the cationic group in the dye molecules. Such a reaction could be favoured at higher temperatures [22].

Effect of initial pH on adsorption process

The pH is one of the most important factors controlling the adsorption of dye onto suspended particles. As the pH increases, it is usually expected that the adsorption also increases [23]. The effect of initial pH of the dye solution on the amount of dye adsorbed was studied by varying pH under constant process parameters (Fig. 3). The removal of victoria blue by perlite increases from 6.2×10^{-6} to 7.4×10^{-6} mol g^{-1} with pH change of dye solution (1.0×10^{-4} mol L^{-1}) from 3 to 6 at 30 ^{0}C. It is remarkable to note from zeta potential and surface charged values that the perlite surface is negatively charged in a wide pH range (3-11) [11]. With increasing pH values the adsorption of victoria blue on perlite tends to increase, which can be explained by the electrostatic interaction of dye cationic species with the negatively charged surface. The electrostatic attraction force of the dye compound with perlite surface is likely to be raised when pH increases [24].
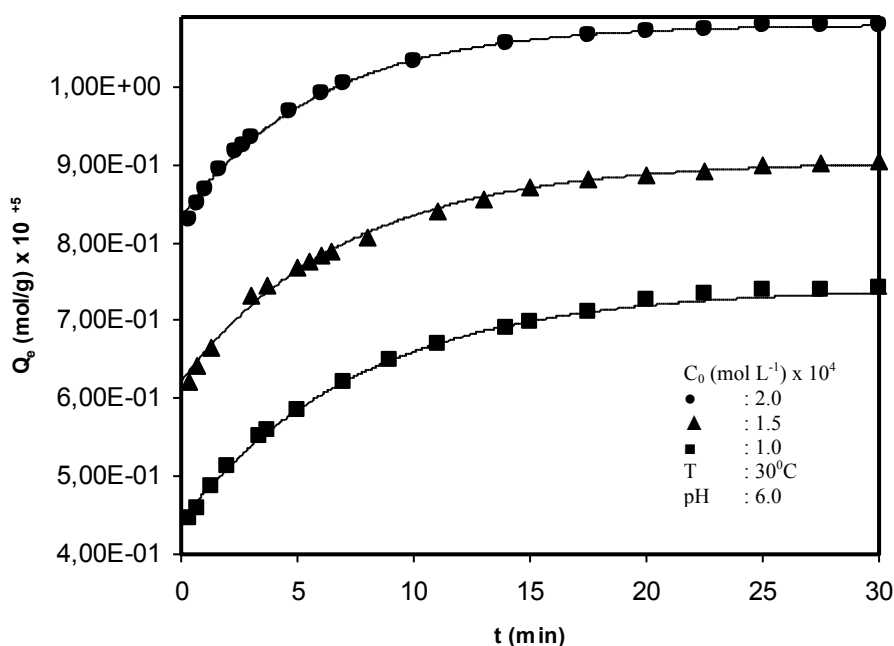
FIGURE 1 - The effect of contact time and concentration on
the removal of victoria blue from aqueous solutions with perlite
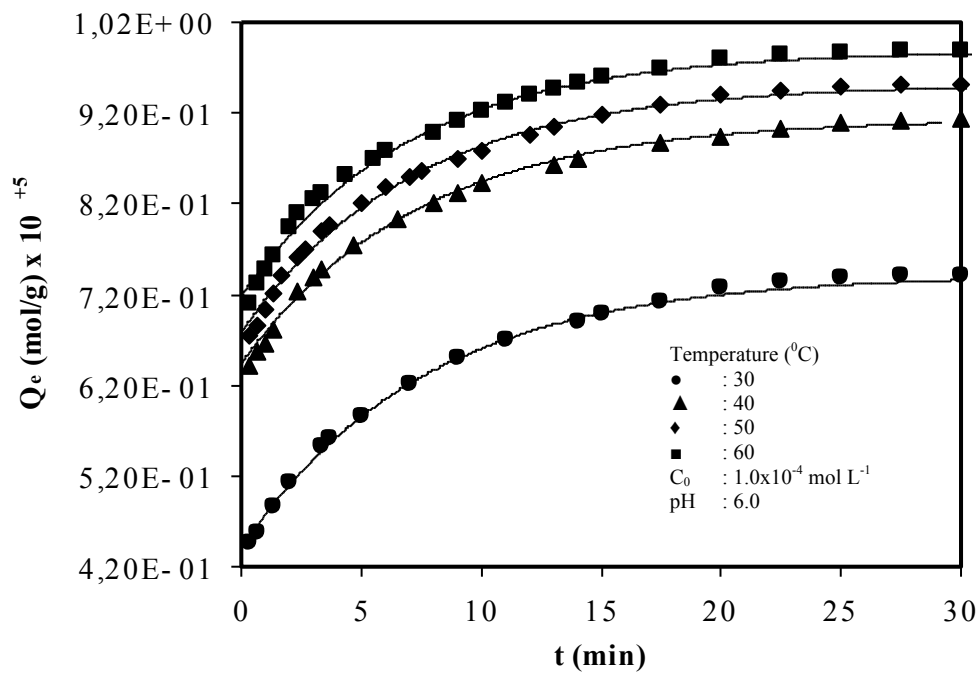
**FIGURE 2 - The effect of contact time and temperatures on
the removal of victoria blue from aqueous solutions with perlite.**
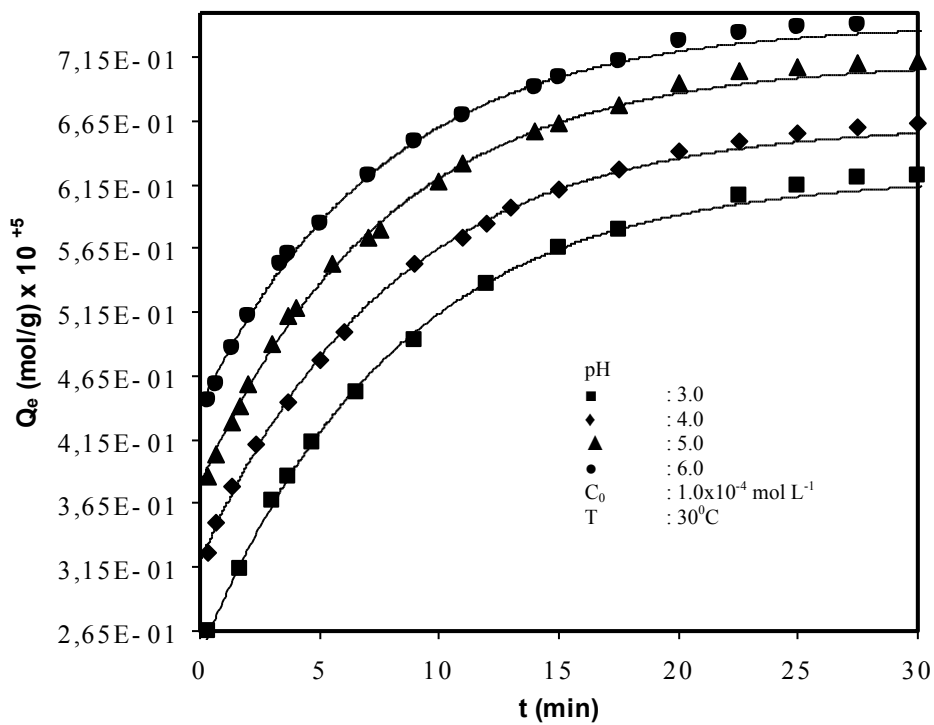
**FIGURE 3 - The effect of contact time and pH on the
removal of victoria blue from aqueous solutions with perlite.**
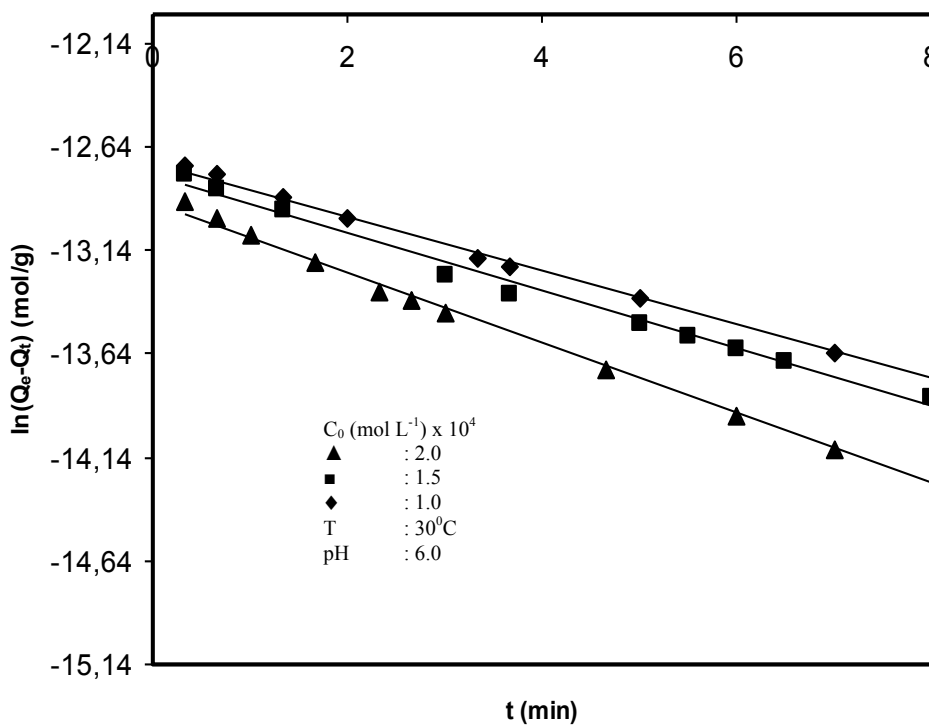
**FIGURE 4 - Lagergren plots for adsorption of victoria blue at
different initial dye concentrations with perlite from its aqueous solutions.**
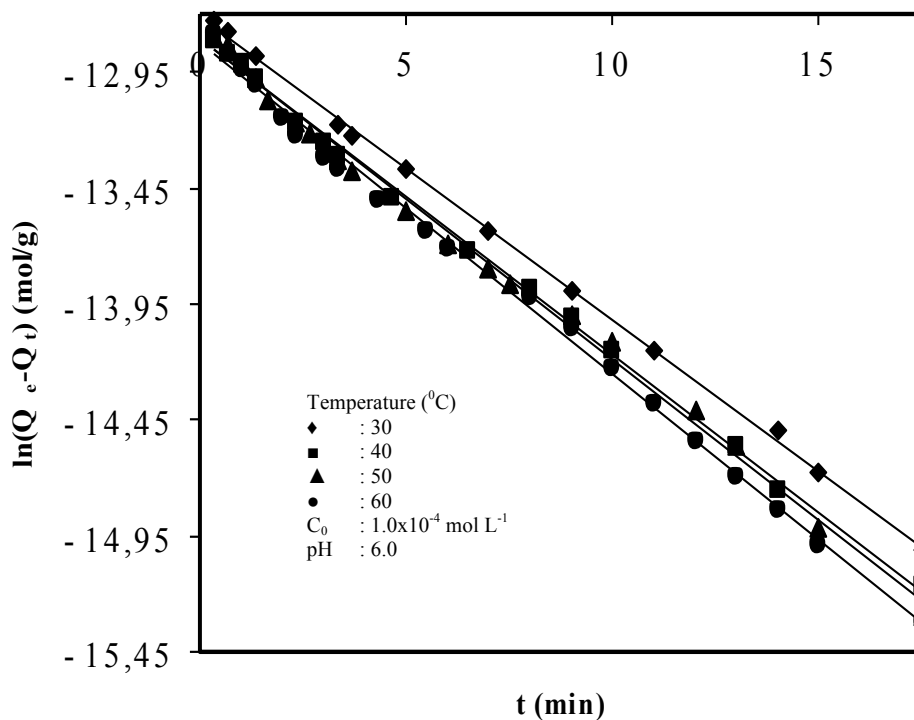
**FIGURE 5 - Lagergren plots for adsorption of victoria blue
with perlite at different temperatures from its aqueous solutions.**
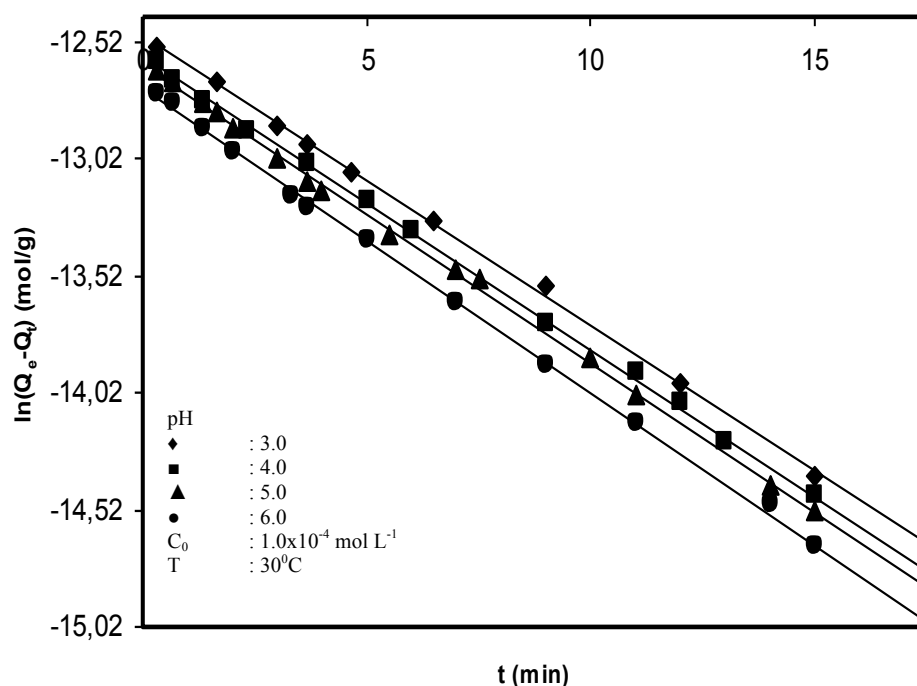
**FIGURE 6 - Lagergren plots for adsorption of victoria blue
with perlite at different pH values from its aqueous solutions.**

Adsorption Kinetics

The plots of Q_e vs t for all the victoria blue-adsorbent systems are found to be exponential indicating the first order nature of the adsorption process (Figs. 1-3). In order to find out whether the adsorption process followed first order kinetics the following generalised first order kinetic equation proposed by Lagergren was followed:

$$\log\left(Q_e - Q_t\right) = \log Q_e - \frac{k}{2.303}t \qquad (1)$$

where Q_e and Q_t are the amount of dye adsorbed per unit mass of the adsorbent (mol g^{-1}) at equilibrium and time t, respectively [1]. The values of $\ln(Q_e-Q_t)$ were correlated with time. The values of first order rate constants and correlation coefficients (r values) are given in Table 2. The fact that all the linear regression coeffcients were higher than 0.99 indicates the applicability of this kinetic equation and the first order nature of the adsorption process of victoria blue on perlite. Lagergren plots are shown in Figs. 4 - 6.

The half-adsorption time of the dye, $t_{1/2}$, i.e. the time required for the perlite to take up half of the amount at equilibrium, is often considered as a measure of the rate of adsorption and for the first order process given by the relationship [1]:

$$t_{1/2} = \frac{\ln 2}{k} \qquad (2)$$

The values of $t_{1/2}$ determined for the tested temperatures are given in Table 2.

**TABLE 2
Kinetics values calculated for victoria blue adsorption on perlite**.

Temp. (^{0}C)	Conc. (M)x10^{+4}	pH	Lagergren equation		$t_{1/2}$ (s)
			r	k (s^{-1})x10^3	
30	1.0	6	0.9995	2.18	319
30	1.5	6	0.9978	2.32	299
30	2.2	6	0.9981	2.52	275
40	1.0	6	0.9989	2.26	306
50	1.0	6	0.9975	2.31	300
60	1.0	6	0.9985	2.38	291
30	1.0	3	0.9996	2.07	334
30	1.0	4	0.9996	2.10	330
30	1.0	5	0.9995	2.14	324

Activation parameters

The activation energy was calculated from the linearized Arrhenius equation (3):

$$\ln k = \ln A - \frac{E_a}{R_g T} \qquad (3)$$

where k is the rate constant (s^{-1}), E_a the activation energy (kJmol^{-1}), R_g the gas constant (J K^{-1}mol^{-1}) and T the temperature (K). A plot of ln k vs the reciprocal of absolute temperature, 1/T, gives a straight line as shown in Fig. 7 and the corresponding activation energy was deter-
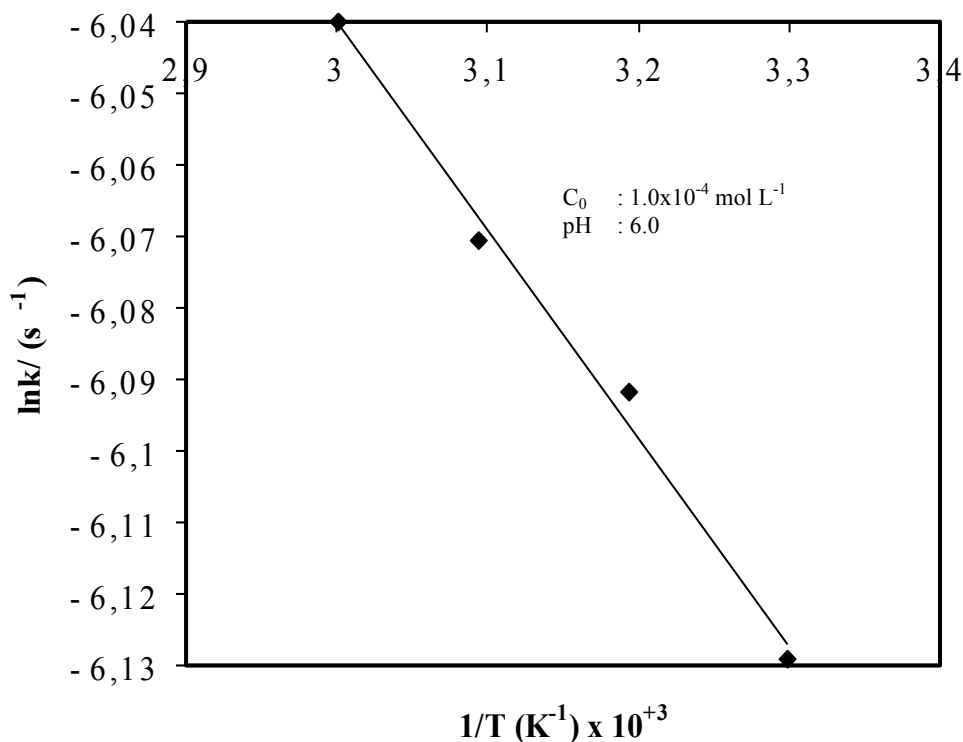
FIGURE 7 - Arrhenius plots for adsorption of victoria blue.

mined from the slope of linear plot. The result obtained was 2.4 kJmol^{-1} (r=0.9957) for adsorption from its solution. As the activation energy is very low, it can be concluded that the process is governed by interactions of physical nature [25].

CONCLUSIONS

Perlite is capable of removing victoria blue from an aqueous solution. The initial rate of adsorption of victoria blue with perlite was high, then followed by a slower rate and gradually approaching a plateau/equilibrium within 30 min. It was found that the rate constant of adsorption increased by increasing the initial concentration, pH and temperatures. The adsorption process was found to be of first order and the value of activation energy as 2.4 kJ/mol.

NOMENCLATURE

Q_e Equilibrium dye concentration on adsorbent, mol g^{-1}.
r   Linear regrassion coefficient.
t   Time, s
T   Temperature, K.
Q_t The amount of dye adsorbed per unit mass of the adsorbent at time, t, mol g^{-1}.

k   Adsorption rate constant, s^{-1}
$t_{1/2}$ The half-adsorption time of dye, s.
E_a Activation energy, kJ mol^{-1}.
R_g Gas constant.

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FEB

A MULTICRITERIA DECISION MAKING METHODOLOGY

FOR SUSTAINABLE ENERGY DEVELOPMENT

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SUMMARY

The annual increase of power energy consumption and the incapability of the currently installed energy production system in a hypothetical area to cover the future demand, are raising the issues of developing new policies and introducing new ways to produce energy in a sustainable way. The purpose of this paper is to investigate how a well-known multicriteria analysis method, namely outranking relation theory, can be used to evaluate alternative scenarios for sustainable management of the energy system in the area. These scenarios are combinations of penetration of different energy producing units, such as conventional units and renewable energy units. A set of sustainability indicators is proposed, that denote resource, environmental, economic and social aspects of each scenario.

KEYWORDS:
multicriteria analysis, sustainable, energy system.

INTRODUCTION

A hypothetical area faces the problem of increasing energy demand that the currently installed system is unable to cover. Therefore, decisions should be made concerning the installation of new power generators and different options are under consideration. These options concern conventional units and renewable energy sources (RES) units according to the availability and the potentials in the area. The main goal is the development to be consistent with the sustainability approach. Therefore, the best scenario should adjust to the environmental protection, the resources availability, the social welfare and the economic viability of the system. Considering these aspects, appropriate sustainability indicators will reveal. In reality the criteria will compete with each other and the goal of multicriteria analysis is to extract the best scenario of producing energy, taking into account all of them. A well-adapted method of multicriteria analysis, namely the fuzzy outranking relation theory, can be proved useful in the many-sited and difficult process of sustainable management.

SUSTAINABLE DEVELOPMENT OF THE ENERGY SYSTEM

In an effort to adopt the principles of sustainable development to the energy system many questions are arising. They mostly concern what should be the best policy, in order to take into account the present needs, but also satisfy our sense of commitment for the future generations. A global and local long-term vision is important. Nowadays, the maturity of the technology can provide energy efficiency and also take advantage of the world's RES. By changing the structure of today's system of energy production, a first step towards sustainability is made, keeping of course, in mind that it is not only the production, which should be changed, but also the current patterns of energy consumption [1]. However, one should not forget the stochastic nature of RES, their high current price and the fact that they are not fairly adaptable in areas where the population is highly concentrated due to their low energy density (they need available space and to be in contact with the natural resources –wind, sun, forests, rural production etc.). Furthermore, construction of RES system inevitably requires non-RES production, such as fossil fuels, which should be taken into account. Decision-making, though, should be well justified. Especially, the electricity market liberalization will initiate major structural changes in energy enterprises [2]. In order to do so, the definition and evaluation of appropriate sustainability indicators will be an essential tool for choosing between alternative future scenarios.

SUSTAINABILITY INDICATORS

Major components of a successful strategy for sustainable development include changing present energy production and consumption patterns, diversifying energy sources and the structure of power production and establishing an energy structure that is less or not at all harmful to the environment. Furthermore, the energy industry is fundamental to the national and global economy, because it is essential for all human activities and determines dramatically their main characteristics. Therefore, the socio-economic development and the improvement of the living standards is of critical importance. Sustainability concept should, therefore, reflect not only the concern about shortage of natural resources and environmental protection, but also the close correlation to the society`s needs for economic development. In order to evaluate different options under sustainability criteria one has to adapt a conceptual approach and focus on those aspects that are directly correlated with the sustainability concept [3, 4]. The first step is to define the appropriate sustainability indicators. They should reflect the following aspects of the system:

- Resources

- Environment

- Society

- Economy

Resources

For a long time the development strategy of civilization had the illusion that energy resources are unlimited. The last decades changes have been witnessed, which are questioning the long-term prospect. After the first and second energy crisis the community at large has become aware of the possible physical exhaustion of fossil fuels. Also it is evident that as much as the fuel consumption is increasing, new technologies aiming to discover new resources are becoming available, leading to a slow increase of the time period for the exhausting of the available energy sources. Whatever the accuracy of the prediction methods is, it is obvious that any inaccuracy in the calculations may affect only the time scale but not the essential understanding that the energy resources depletion process has begun and requires the human action before it's too late. Appropriate resource indicators can be for example: the oil consumed/kWh, carbon consumed/kWh, and other materials (aluminium, steel) consumed/ kWh.

Environment

Environmental problems and energy production/ consumption are closely linked. Global threat to the climate is broader than a simple environmental problem. Energy production is the main source of greenhouse gases in the atmosphere, accounting to about 85% of gas emissions in developed countries and to a smaller but growing share in developing countries. Both near and long-term trends show significant increases in energy-related carbon dioxide emissions. Projects through 2010 suggest that without the adoption of new policies, emissions could rise 30% above the 1999 level. Technology has a critical role to play in reducing energy-related greenhouse gas emissions and a technology strategy must focus simultaneously on the short- and long-term. Appropriate environmental indicators can be for example: CO_2 produced/ kWh, SO_2 produced/ kWh, NOx produced/ kWh, and waste produced/ kWh.

Society

Each energy policy affects the society in different ways. Social development needs both economic growth and a healthy environment. One of the most important figures, which should be taken into account, is the number of jobs to be opened. For a society with a high unemployment rate this can be very important and also the scientific research opportunities for new scientists is a sign for the sustainable development of the society. A society that confronts its members with high long- or short-term risks cannot be considered as sustainable. Appropriate social indicators can be, for example, number of new jobs to be opened/ kWh, number of working days lost/ kWh.

Economy

Economic growth requires a secure and reliable energy supply. This is an aspect that can be considered from two different levels. First, the impact each option has on the local economy and second, the impact on the company that is responsible for the production and contribution of electric energy. Regardless of public or private character of the company, the aim is to be viable and profitable. If energy prices are raised too quickly in an effort to combat environmental concerns, energy may become too costly and, thus, placed beyond the reach of those who need it most. Lower fuel prices widen the access to energy, but also encourage inefficient utilization of energy resources and accelerated resource depletion. Appropriate economic indicators can be, for example, the economic profit of the system which can be considered to be the sale price minus the cost of production/ kWh, and economic profit of the local society which can be considered to be: number of workers x average wage/kWh.

ALTERNATIVE SCENARIOS FOR ELECTRICITY ENERGY PRODUCTION

The need for an active involvement in allocating scarce, RES and non-RES, for the achievement of a sustainable future has been obvious. Taking into consideration that the annual consumption of electric energy in the hypothetical area will increase, the question that arises is: what are the most promising combinations of different energy producing units penetration, such as conventional

units and RES units. The alternative scenarios under investigation and their assessment may reveal the appropriate policies and aspects of steps that should be followed towards sustainability. Different units to produce the necessary extra amount of electric energy for the area under consideration are conventional units (crude oil, diesel, liquid gas) and RES (wind energy, solar PV, biomass, hydroelectric energy).

Possible scenarios that one could wish to evaluate and compare, based on the sustainability indicators that we have selected and for which data are available, can be the following:

- **Scenario A -** conventional vs. RES (The penetration of conventional sources is > 50%)

- **Scenario B -** conventional and RES are equal (penetration of both conventional and RES is 50%)

- **Scenario C -** RES vs. conventional (The penetration of RES is >50%)

It is aimed to be able to establish comparisons under the reliability of a highly adaptable way, namely, under a multicriteria decision – making methodology.

MULTICRITERIA DECISION MAKING METHODOLOGY

The general multicriteria analysis problem consists in choosing the best action, in a finite set of actions A= {a, b, c...}, evaluated according to n criteria, noted as g_1, g_2,..., g_n. The criteria are defined as real applications of type g_i: A→$G_i \subseteq$ R, in such a way that for a given action a, the real number $g_i(a)$ represents the performance of action a for the ith criterion. The higher the $g_i(a)$, the more this action satisfies the preferences of the decision-maker. For two actions a and b, if $g_i(a)=g_i(b)$ one says that the relation is indifferent. In the present case, set A is the set of alternative scenarios of electric energy production and the criteria are appropriate sustainability indicators. Moreover, for the purpose of the method each criterion is given a weighting factor p_i. These weights p_i reflect the importance of each criterion and they sum to unit. The assessment procedure explored is based on fuzzy outranking relation theory. Apart from classical relations of preference and indifference, an outranking relation allows to take into account incompatibilities between actions. Furthermore, the establishment of a fuzzy outranking structure provides a more realistic view of pairwise comparisons, since it takes into consideration the uncertainty on the criteria evaluation [5]. Fuzzy outranking relation in AxA is called a function d:AxA→[0, 1] in which the different values d(a,b) denote the strength of the relationship

between any two actions a and b in A, as described in [6, 7].

More specifically:

- a is preferred to b ⇔ d(a, b) > d(b, a)

- a is indifferent to b ⇔ d(a, b)=d(b, a)>0

- a is incomparable to b ⇔ d(a, b)=d(b, a)=0

To obtain an explicit form for d (a, b), the first are defined n pairs of partial fuzzy relations, $d_i(a, b)$ and $D_i(a,b)$, where i=1,2,…n, and are relative to each criterion. The first, fuzzy relation $d_i(a, b)$ is the partial fuzzy outranking relation of b by a for the ith criterion. The second, fuzzy discordance relation $D_i(a,b)$, designates the discordance degree of criterion i for pair (a,b).Then the method assesses fuzzy outranking relation by using fuzzy partial relations.

For more than two alternatives, a supplement method exists for ranking. One can calculate for each alternative an indicator that reflects the non-domination degree of the alternative simultaneously by all the others [7]. This indicator, which varies from 0 to 1, is given in the formula below:

$$\mu^{ND}(a) = 1 - \max_{b \in A}[d(b,a) - d(a,b)]$$

The result is that the higher the non-domination degree, the more preferable the alternatives. The non-domination degrees are not utilities that can result in ranking over A, but merely represent priorities of choice, especially if the values are close to unit. Furthermore the actions a for which μ^{ND} (a)=1, define the set of non-dominated actions.

The algorithm below describes the steps of the method.

Algorithm

```
for a:=1 to Number of Actions do begin
 for b:=1 to NumberOfActions do begin

  C[a,b]:=0;
  for i:=1 to Number of Criteria do begin

  {calculation of partial fuzzy outranking relations}
   if (g[i,b]-g[i,a])<=0  then dp[i,a,b]:=1
        else if (g[i,b]-g[i,a])>=S[i]  then dp[i,a,b]:=0
            else dp[i,a,b]:= 1-((g[i,b]-g[i,a])/S[i]);

  {calculation of fuzzy discordance relations}
  if (g[i,b]-g[i,a])>=U[i]  then D[i,a,b]:=1
        else if (g[i,b]-g[i,a])<=S[i]  then D[i,a,b]:=0
            else D[i,a,b]:= ((g[i,b]-g[i,a]-S[i])/(U[i]-S[i]));

  {calculation of fuzzy concordance relations}
  C[a,b]:= C[a,b]+ dp[i,a,b]*P[i];
```

```
end; {for i}
{calculation of fuzzy outranking relations}
    product:=1;
     for i:=1 to Number of Criteria do
   if C[a,b]< D[i,a,b] then product:=product*(1-D[i,a,b]);
   if product=1 then d[a,b]:=C[a,b]
   else d[a,b]:= (C[a,b]/(1-C[a,b]))*product

 end; {for b}

end; {for a}

{calculation of the Non-Domination Degree Index}
for a:=1 to Number of Actions do begin
 max:=0;
  for b:=1 to Number of Actions do
  if max<(d[b,a]-d[a,b]) then max:=d[b,a]-d[a,b];
 uND[a]:=1-max;
end; {for a}
```

The following data structures are used to bring input to the algorithm:

- g[i,a] a 2 dimensional matrix of size GxA, for the criteria $g_i(a)$

- P[i] a 1 dimensional matrix of size G, for criteria weights P_i

- S[i] a 1 dimensional matrix of size G, for maximum non-significant thresholds S_i

- U[i] a 1 dimensional matrix of size G, for veto-thresholds U_i

The following data structures are used to hold the partial and final outputs of the algorithm:

- dp[i,a,b] a 3 dimensional matrix of size GxAxA, for partial fuzzy outranking relations $d_i(a,b)$

- D[i,a,b] a 3 dimensional matrix of size GxAxA, for fuzzy discordance relations $D_i(a,b)$

- C[a,b] a 2 dimensional matrix of size AxA, for fuzzy concordance relations $C(a,b)$

- d[a,b] a 2 dimensional matrix of size AxA, for fuzzy outranking relations $d(a,b)$

- uND[a] a 1 dimensional matrix of size A, for Non-Domination Degree Indicator μ^{ND}

where G is the number of criteria and A the number of actions.

Example: Let us assume an energy system that can be characterized by 4 criteria

- g_1 : The amount of oil consumed for the operation and construction of the energy production unit

- g_2 : The amount of CO_2 produced by the energy productive unit at operation but for the construction of the unit

- g_3 : Economic profit of the system: average price of the energy unit sold, minus the cost of production

- g_4 : Number of new jobs to be opened.

Let also assume 2 hypothetical different actions evaluated, based on the above 4 criteria. Each action consists of a different combination of Energy Producing Unit penetration (RES and conventional).

TABLE 1
Multicriteria evaluation of the different scenarios A and B.

	g_1	g_2	g_3	g_4
A	5.1	5.4	3.5	2.5
B	7.2	4.3	3.8	1.9

For the method to be applied the following are needed:

- The weights p_i, are set arbitrarily 0.25 given the same importance to each criterion.

- Very small differences $g_i(a)-g_i(b)$ are considered non-significant. We need maximum non-significant thresholds for each criterion and we set arbitrarily $s_1=2$, $s_2=2$, $s_3=2$ and $s_4=2$

- Very big differences correspond to incomparability. We need veto thresholds and we set arbitrarily $v_1=5$, $v_2=5$, $v_3=5$, $v_4=5$.

Now we proceed to the necessary calculations and the results are shown in Tables 2-6.

TABLE 2
Partial fuzzy outranking relation d_i(a, b).

d_1(a, b)	A	B
A	1.000	0.000
B	1.000	1.000

d_2(a, b)	A	B
A	1.000	1.000
B	0.450	1.000

d_3(a, b)	A	B
A	1.000	0.850

B	1.000	1.000

$d_4(a, b)$	A	B
A	1.000	1.000
B	0.700	1.000

TABLE 3
Fuzzy discordance relation $D_i(a, b)$.

$D_1(a, b)$	A	B
A	0.000	0.033
B	0.000	0.000

$D_2(a, b)$	A	B
A	0.000	0.000
B	0.000	0.000

$D_3(a, b)$	A	B
A	0.000	0.000
B	0.000	0.000

$D_4(a, b)$	A	B
A	0.000	0.000
B	0.000	0.000

TABLE 4
Fuzzy concordance relation C(a, b).

$C(a, b)$	A	B
A	1.000	0.713
B	0.788	1.000

TABLE 5
Fuzzy outranking relation d (a, b).

$d(a, b)$	A	B
A	1.000	0.713
B	0.788	1.000

TABLE 6
Non-domination degree index for each Scenario (action).

$\mu^{ND}(a)$	A	B
	0.925	1.000

It can be concluded according to the above that action B is preferred to action A since d(B, A) is greater than d(A, B). Furthermore, the non-domination degree of action B is equal to 1.

CONCLUSIONS

This paper is aimed at suggesting a methodology of decision-making for the electricity generation system extent in a hypothetical area. A multicriteria analysis meth-od, namely fuzzy outranking relation theory, is shown to be a powerful tool for denoting the strength of relation and, therefore, providing information to the decision-makers to choose between possible future scenarios. The assessment criteria are appropriate sustainability indicators, which reflect the economic, social, environmental and resource aspects of each scenario. The method deals with fuzziness at the evaluation of the criteria and also takes into account the specific weight of each criterion, which can vary under different priorities and circumstances.

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AIRBORNE CONCENTRATIONS OF ASBESTOS DURING REMOVAL OF PIPE/BOILER INSULATION USING GLOVEBAGS WITH AND WITHOUT CONTAINMENTS

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SUMMARY

This investigation studied personal airborne exposure to asbestos during two different projects involving pipe/boiler insulation abatement, one employing glovebags alone and one employing glovebags used inside two different containment systems (critical barriers and full containment with critical barriers). Asbestos floor tile was also abated during one of the projects. Results suggest that higher exposure levels are observed when asbestos abatement is conducted with glovebags alone. Personal exposure levels when using glovebags with or without containment systems did not exceed the United States Occupational Safety and Health Administration Permissible Exposure Limit (PEL) for airborne asbestos. Based on the summary statistics for all projects, probability estimates and upper confidence intervals for sampling data, there is a low likelihood of exceeding the PEL during removal of pipe/boiler insulation, using glovebags with and without containments, and floor tile. All pipe/boiler insulation and floor tile abated contained asbestos that exceeded the United States Environmental Protection Agency definition of asbestos-containing material.

KEYWORDS:
asbestos abatement, asbestos-containing material, management of asbestos, engineering controls, legislating science.

INTRODUCTION

Asbestos abatement has emerged as a major industry in many countries [1-3]. Removal or abatement of asbestos-containing materials (ACM) is regulated by various governmental agencies in the United States [4, 5]. The purpose of these regulations is the protection of the environment, persons occupationally exposed and public health [5]. Previous studies [6, 7] have suggested that with the employment of reasonable engineering controls and work practices the airborne exposure levels of asbestos abatement to workers are commonly below the US Occupation Safety and Health Administration (OSHA) Permissible Exposure Limit (PEL) of 0.1 fibers per cubic centimeter (f/cc)-Time Weighted Average (TWA) [4]. Thus, when appropriate practices are employed there is little need for employment of various types of personal protective equipment (PPE) including respirators [6]. Use of PPE, including respirators, makes abatement work more difficult for these workers and increases stress [6]. Respirator use also reduces the efficiency of work activities. This emphasizes the importance of knowing the exposure levels to workers, so appropriate PPE can be matched with employed work practices.

This study evaluated the use of glovebags inside two different types of containments (full containment with critical barriers and critical barriers alone) and without a barrier. The ACM abated were pipe/boiler insulation. These data provide information on exposure levels during the abatement of pipe/boiler insulation when using glovebags alone and glovebags in different containment structures. A small amount of asbestos floor tile was removed as part of one project. Data on personal exposure during the removal of floor tile are presented.

MATERIALS AND METHODS

Personal air samples [6] were collected from workers removing asbestos pipe/boiler insulation and floor tile in two different projects. The percent and type of asbestos were determined by polarized light microscopy [8]. The US Environmental Protection Agency (EPA), identifies a material as ACM if its asbestos concentration is greater than 1% [9]. Abatement was conducted in the mid-Atlantic region of the United States in 2002. Project one was about 6 calendar days in duration and project two was about 10 calendar days in duration. Floor tile was removed as part of project one only. The quantity of asbestos removed was: project one insulation 2,000 linear feet (lf), floor tile 1,100 square feet; and project two insulation 1,900 lf. Floor tile and insulation were not removed at the same time.

Abatement was conducted following the methods required by OSHA [4]. Project one employed glovebags alone and glovebags with critical polyethylene plastic barriers. Critical barriers covered windows, doors and other openings but not walls and related structures. Project two consisted of glovebags alone and both critical barriers and full containment. Full containment consisted of polyethylene plastic covering all surfaces in the work area, including walls and doors. Both projects consisted of a negative pressure system using negative air machines with HEPA filtration (NAM). Floor tile was removed under full containment and NAM. All projects employed wet methods during removal [4]. Each project had a three stage decontamination chamber [9].

Personal air samples were collected as task-length averages (TLA) and excursion limits (EL). TLA samples were determined for only the time period (about one to three hours) of sampling, and were not adjusted for a period of eight hours (TWA). EL samples are those collected for a 30 minute time period. OSHA defines an exceedance of the EL to be 1.0 f/cc-30 minutes/day [4]. Samples were collected using 25-mm diameter electrically conductive extension cowl cassettes with mixed ester filter membrane operated open face [4]. These samples were collected at 2 lpm (nominal) using a personal air sample pump in the breathing zone of the worker [6]. The NIOSH 7400 method was used for analyzing filters [6]. This method employs phase contrast microscopy (PCM). Blank cassettes were analyzed and no fibers were observed on these filters. Final clearance was performed for these projects employing PCM and the clearance level was <0.01 f/cc.

Exposure results were reported as summary statistics [6, 7]. Statistical comparison of glovebags alone and glovebags within a barrier system was performed using the Wilcoxon Rank Sums test. Outliers were determined using the Grubbs test on data transformed using natural logarithms and those non-transformed. Confidence intervals (CI), at 95%, were determined using a method for non-normal populations [10]. Sample results below the reportable detection limit were included in calculations at one-half the reported value [11]. Probability (confidence coefficient), using TLA, of at least 5% of workers exceeding the OSHA PEL was determined using a graphic method [12]. Potential of exceeding the PEL was calculated using the TLA for each sample. Statistical significance was defined at 5% and all calculations were performed at the 95% level.

RESULTS AND DISCUSSION

The approximate asbestos content of pipe/boiler insulation at projects one and two were both 45%. Floor tile was reported as having 13% asbestos. All asbestos was chrysotile and pipe/boiler insulation became friable upon its removal/disturbance. All removal areas passed final clearance after the work was completed.

Personal exposure results during abatement of pipe/boiler insulation and floor tile are shown in the Table. No exposure value exceeded the OSHA PEL or EL for airborne asbestos. All EL samples were <0.045 f/cc-30 minutes/day except one sample in project one which involved glovebag alone and that value was 0.049. There were no outliers for either transformed or non-transformed data. Previous studies [13-15] reported environmental and occupational exposure, including airborne asbestos [6], are non-normally distributed and best fit a logarithmic distribution.

Probability of overexposure, which employed arithmetic means, of OSHA's PEL was less than 5% for each study (project one, two and floor tile) except for glovebag alone in project one which was 10%. These data suggest a low likelihood of exceeding the OSHA PEL during these abatement practices. The lack of any single sample exceeding the OSHA PEL and no CI for any set of data having an upper value above this regulatory limit, support these probabilities.

Previous studies [6, 7, 16, 17] have reported similar exposure levels for abatement of pipe/boiler insulation and floor tile. Since the NIOSH 7400 method counts all fibers [8], the airborne concentration of asbestos is most likely much lower than that reported in the Table [18, 19]. Some have suggested using a value one-half of the reported concentration by the 7400 method for estimating the asbestos level [6]. During monitoring at the World Trade Center for airborne asbestos, OSHA reported in one of their sampling tables only asbestos like materials [subtracting "obvious non-asbestos fibers" (ONAF)] in concentrations of airborne samples [19, 20]. This asbestos concentration (subtracting the ONAF) was only a small fraction of the actual total fibers reported with the majority of samples having no asbestos fibers [19, 20]. Some suggest that this has signaled a change in the methodology accepted by OSHA in reporting asbestos fiber concentrations by PCM [19, 21, 22].

In the present study, there was both significance and non-significance for glovebags alone compared to glovebags in containments (Table). However, since no single exposure value exceeded the OSHA PEL nor is there a likely probability of such occurrence, any statistical difference is considered a false positive (type II error) result [17]. The statistical difference for glovebags in full containment and the lack of difference associated with critical barriers

**TABLE - Personal sample summary statistics for boiler/pipe insulation abatement
using glovebags alone and in containment and/or critical barriers, in f/cc.**

Type of Sample	Number of Samples	Arithmetic Mean	Geometric Mean	Standard Deviation	Geometric Standard Deviation	Range
Containment[1][+]	15	0.007 (0.046)	0.006	0.002	2.1	<0.008-<0.015[3]
Glovebag alone[+]	11	0.016 (0.055)	0.009	0.009	2.0	<0.009-0.031
Containment2*	12	0.006 (0.001)	0.006	0.003	1.9	<0.002-<0.0224
Glovebag alone*	51	0.003 (0.005)	0.016	0.018	2.9	<0.002-0.090
Floor tile	4	0.005 (0.004)	0.004	0.004	2.2	<0.003–0.01

1 glovebag with critical barriers; () is CI value at 95%; 2 full containment and critical barrier; + Without and with glovebags samples were not statistically different ($p > 0.05$) and were for the same project; * Without and with glovebags samples were statistically different at less than 5% ($p < 0.05$) and were for the same project; 3 highest reportable value was 0.009 f/cc; 4 highest reportable value was 0.008 f/cc; all values are TLA; number of values below detection limit for individual samples for 1+containment, +glovebag alone, 2* containment, *glovebag alone, and floor tile were 12, 4, 13, 11, and 2, respectively.

is suggested to be chance. Since there was a large number of values below the detection limit this will have a strong influence on statistical relationships. Statistically lower exposure levels for glovebag operations inside containments as compared to glovebags alone have been reported in one study [1]). This previous study [17] of glovebags with and without containment also suggested that a type II error exist for those exposure data. When ONAF are considered in these data, it is likely that there is no difference in actual asbestos concentrations. Any observed concentration difference between glovebag alone and glovebag with containment is likely a result of air exchanges in the work area from use of NAM.

Numerous studies [6, 7] during abatement of asbestos floor tile have reported exposure levels below the OSHA PEL. Data presented in this investigation support categorizing floor tile in a separate grouping as compared to other forms of friable asbestos. The floor tile industry did receive concessions from OSHA in lessening the requirements of abatement [23]. However, these requirements dictate removal of whole pieces of tile. The purpose of whole piece removal was to ensure low exposure levels during abatement. Based on the data presented here and elsewhere [6, 7], even when floor tile is broken exposure levels are well below the OSHA PEL. When the ONAF method is considered along with previous exposure results, it is highly unlikely that the PEL or EL will ever be exceeded regardless of work practices employed.

Exposure values for this work suggest that there is little requirement for employment of respirators [6]. With great frequency, at least in the United States, specifications require employment of respirators without regard to historical data associated with the type of material being abated and work practices being implemented [6]. It has been suggested that use of respirators at levels below the OSHA PEL may have more detriment than benefit [6, 7]. This demonstrates the requirement of appropriate selection of PPE in relation to the type of activities being undertaken.

Regulatory standards for asbestos abatement have been established on the basis of whether the material is defined as ACM [6]. It has been suggested that requirements be instead based on the anticipated exposure levels. This would provide more flexibility for controls and work practices as related to potential exposure than one requirement meeting all conditions. The current criteria for regulating asbestos abatement have been referred to as a form of legislating science [6, 7, 17]. By establishing requirements based on exposure levels this will result in a more scientific approach to abatement controls.

When employing glovebags, there appears to be no exposure benefit associated in employment of critical barriers or full containment systems. Thus, establishing barriers for glovebag activities will only add additional cost for this work. Any regulatory or contractual requirements that establish containments for glovebag activities, based on these and other data [17], provide no exposure benefit to workers and are a form of legislating science [6, 7].

These data show that when good engineering controls and work practices are employed exposure levels to workers are low. In addition, various types of asbestos abatement (e.g. floor tile, window caulking) result in low exposure concentrations regardless of the practices employed [6, 7]. These data suggest that abatement requirements should be based on the type of material being abated along with the practices employed as they relate to exposure. The importance of eliminating the legislating of science for asbestos abatement has been clearly demonstrated [6, 7, 20]. Additional studies are warranted on exposure levels for different types of abatement practices and materials. These studies should include the relationship of various abatement practices with anticipated exposure levels.

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SUPERCRITICAL FLUID EXTRACTION AND GC DETECTION OF p,p¹-DDE AND PCB CONGENERS IN SOME SAMPLES OF DYING SPECIES FROM SARDINIA

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SUMMARY

In an environmental pollution research some wild animals, deceased due to accidental causes, were collected from all over Sardinia. In order to detect the possible residues of unknown pollutants the isolated livers of these animals, previously lyophilized, were analyzed. First, by a GC-MS method, we identified the p,p¹-DDE and some PCBs belonging to 5, 6, 7, 8 series PCBs.

Later we set up a method for quantification of these compounds, using chicken liver as a model, by GC-ECD. The spiked chicken liver, free from p,p¹-DDE and PCBs, was lyophilized and extracted by supercritical fluid extraction (SFE), subsequently the solutions obtained were subjected to cleanup by solid phase extraction (SPE). Finally, this method was employed to quantify p,p¹-DDE and PCBs in the samples of the specimens collected.

KEYWORDS: Extraction methods; environmental analysis; liver; GC-MS; GC-ECD.

INTRODUCTION

Chlorinated hydrocarbon contaminants such as polychlorinated biphenyls (PCBs) and 1,1-dichloro-2,2-bis (4-chloro-phenyl) ethene (p,p¹-DDE), a metabolite of the pesticide DDT, are well-known global environmental pollutants which accumulate in biological matrices [1] such as liver of wild mammalians and birds, particularly raptorial birds [2, 3]. PCBs are predominant environmental contaminants. They can be found in different areas all over the world and are very persistent compounds. Their properties suggest that they are a probable threat to health, particularly for the wild animals [4, 5]. p,p¹-DDE has not been registered in many states for about 25 years; however, because of its high stability and persistence in the environment, effect levels for different biological matrices, such as brain and liver, have been reached in many animal specimens [6-11]. The effects of PCBs and p,p¹-DDE on humans have been investigated and many effects were observed such as liver damage, respiratory disorders, severe ocular signs, reproduction disorders, damage to the endocrine system, various neurological symptoms, immunodeficiency and dermal lesions [12-14].

In an environmental pollution research, 51 specimens, some of them belonging to dying species, deceased from accidental causes were collected from all over Sardinia. In order to detect the possible residues of unknown pollutants, their isolated livers were previously lyophilized and extracted by supercritical fluid extraction (SFE), using CO_2 as solvent. The selective extraction of the analytes from the lipid containing material was extremely difficult because supercritical CO_2 readily solubilized free fatty acids, glycerides and sterols. Since co-extraction of the lipid matter was unavoidable and adversely affected the separation and detection of the analytes, a cleanup procedure was required before the analysis. Therefore, we have employed a solid-phase extraction (SPE) procedure using a C_{18} cartridge to remove the interfering substances. The solutions obtained were analyzed using a quadrupole mass detector (GC-MS). In this way we have identified the p,p¹-DDE and eleven polychlorinated biphenyl compounds belonging to 5, 6, 7, 8 PCBs series (Table 1).

The procedure for quantification of DDE and PCBs was conducted using chicken liver as a model and four PCBs, one for every series, as standards (Table 2).

Chicken liver is reported in literature as a suitable matrix for determination of PCB congeners by SFE [15]. The spiked chicken liver, free from p,p¹-DDE and PCBs, was lyophilized and extracted by SFE and cleaned by SPE (as above). The solutions obtained were analyzed by capillary gas chromatography using an electron capture detector (GC-ECD) in order to quantify the residues of

TABLE 1 - Retention times (t_R, min.) and characteristic mass fragment ions for pollutants [relative abundances (%) is given in parentheses].

Pollutant	M.W.	t_R (ECD)	t_R(MS)	m/z (relative abundance)
DDE	316	9.17	11,27	246 (100), 318 (68.4), 248 (63.7)
5-Cl PCB	324	9.60	12.61	326 (86.4), 328 (57.5), 324 (57)
5-Cl PCB	324	11.16	14.17	
6-Cl PCB	359	11.70	14.89	
6-Cl PCB	359	11.87	15.10	360 (100), 362 (81.3), 358 (53.6)
6-Cl PCB	359	12.68	15.86	
7-Cl PCB	394	13.57	16.66	
7-Cl PCB	394	13.72	16.77	394 (100), 396 (97), 398 (48)
7-Cl PCB	394	14.07	17.64	
7-Cl PCB	394	14.75	18.11	
8-Cl PCB	429	15.30	18.33	430 (100), 428 (85.6), 432 (66.7)
8-Cl PCB	429	15.85	18.98	

TABLE 2 - Full names of the PCB standards used in this study.

IUPAC N°.	Series	Congener
PCB-82	5 – Cl	2,2',3,3',4 – pentachlorobiphenyl
PCB-128	6 – Cl	2,2',3,3',4,4' - hexachlorobiphenyl
PCB-170	7 – Cl	2,2',3,3',4,4',5 - heptachlorobiphenyl
PCB-194	8 – Cl	2,2',3,3',4,4',5,5' - octachlorobiphenyl

the p,p^1-DDE and PCBs. The calibrations graphs were obtained spiking blank extracts of chicken liver with 2–bromo–8–nitronaphthalene as internal standard. The linearity of calibration curves was studied over an appropriate concentration range. The recovery of the method was studied at six and eight different fortification levels for the PCBs and p,p^1-DDE, respectively. Least – squares and regression analysis of the data obtained shows that the relationships between "added" and "found" were quite satisfactory. Finally, this method was employed to quantify the p,p^1-DDE and PCBs in the liver of the specimens collected.

EXPERIMENTAL

Standards and chemicals

The PCBs standards (99%) and p,p^1-DDE (99%) were purchased from Dr. Ehrenstorfer GmbH (Germany); PCBs chemical names are reported (Table 2) on the basis of the IUPAC derived numbering system [14]. 2-Bromo-8-nitro-naphthalene (98%) was purchased from Ega Chemie (Germany). CO_2 (purity 99.998%) was purchased from SIAD s.p.a. (Italy). SPE cartridge Chromabond C18 was purchased from Macherey-Nagel (Germany) (500 mg/6ml). All other reagents were of analytical grade and were purchased from Carlo Erba Reagents (Milan Italy).

Instrumentations

The livers were homogenized with a blender (Commercial Bnendor) and lyophilized with an Edwards Modulyo apparatus. The SFE extractions were carried out on a Hewlett-Packard 7680 T controlled by PC Compaq 486. The GC-MS analyses were performed on a Hewlett-Packard 5985 mass spectrometer equipped with an HP 5890 series II gas chromatograph coupled with an HP 5988 GC/MS direct interface. The GC-ECD analyses were performed on the same above described chromatograph equipped with an ECD detector.

Liver samples

Liver samples of deceased specimens were collected from personnel of Istituto di Patologia Generale, Anatomia Patologica e Clinica Ostetrico – Chirurgica Veterinaria, University of Sassari (Italy) and stored at – 30 °C. Chicken liver, free from p,p^1-DDE and PCBs, was purchased always from the same chicken farm (Mura, Sassari, Italy) and stored at –30 °C.

Sample preparation.

Primary standard stock solutions of p,p^1-DDE and each one PCB (10 – 0.01 µg ml^{-1}) were prepared in isooctane. Internal standard, 2-bromo-8-nitro-naphthalene (IS), stock solution (0.01 µg ml^{-1}) was prepared in isooctane. Calibration standard samples were prepared spiking the

extracts of blank liver samples with the appropriate volumes of primary and IS standard solutions. For recovery determination, six liver samples with PCBs (10, 5, 1, 0.5, 0.1, 0.05 µg g^{-1}) and eight with p,p'-DDE (10, 5, 1, 0.5, 0.1, 0.05, 0.01, 0.005 µg g^{-1}) were spiked by adding the appropriate volumes of standard stock solutions (Table 3). Quantitative data were obtained adding the appropriate volume of IS stock solution in the sample solutions obtained from extraction and cleanup procedure before injection.

TABLE 3
Recovery data of spiked chicken liver samples (six replicates).

pollutant	Mean rec. %	Mean SD %
PCB-82	82	3.9
PCB-128	81	5.2
PCB-170	79	4.4
PCB-194	78	4.2
DDE	92	5.9

SFE and SPE cleanup procedure.

3g of chicken liver was weighed and spiked with an appropriate volume of standard solution to obtain the quantities reported in Table 3. The liver was homogenized with a blender and subsequently lyophilized. The SFE of the pollutants (PCBs and p,p'-DDE) from the lyophilized was begun at 150 atm. and 50 °C (10 min. static), continued at 200 atm. and 60 °C (10 min. dynamic), and finished at 250 atm. and 70 °C (10 min. dynamic) using carbon dioxide only. Collection of the extracted material was performed with 6 ml of methanol (flow 1 ml/min). The methanol solution from the off-line extraction was evaporated to dryness under nitrogen steam at room temperature and then reconstituted with 10 ml of diethyl ether.

In order to remove the interfering substances, the ether solution was eluted in a Chromabond C$_{18}$ cartridge (500 mg/ 6ml) previously conditioned with ethyl acetate (8 ml) first, then with dichloromethane (8 ml). The collected ether solution was evaporated under nitrogen steam at room temperature until just dry. The residue was redissolved in isooctane, IS in isooctane solution was added and the final volume was adjusted to exactly 10 ml. A 10 µl of the final solution was then analyzed by GC with ECD detector.

In the same way, as described above, the solutions obtained from livers of specimens were analyzed by GC-MS and, after adding the IS, quantified by GC-ECD.

Instrumental analysis
GC-MS

The solutions obtained by extraction from livers of specimens deceased were analyzed by GC-MS in the electron impact (EI) mode. The GC was fitted with a fused silica capillary column coated with HP5 cross linked methyl silicone (Hewlett-Pakard, USA; 30 m, 0.53 mm i.d., 0.88 µm film thickness). The oven temperature program was as follows: initial temperature held at 80 °C for 1 min., 30 °C/min. to 190 °C, and 6 °C/min. to 300 °C (2 min.). The transfer line, ion source and quadrupole temperatures were 250 °C, 230 °C and 100 °C, respectively. The carrier gas helium was of ultra–high purity and the electron impact was 70 eV. The ions were scanned in the selected ion monitoring (SIM) mode. The main fragments obtained and their relative abundances are shown in Table 1. The data are in good agreement with the different diagnostic ions for these compounds [16].

GC-ECD analysis

The GC column and the oven temperature program were the same as that used for GC-MS. A 2 µl volume was used for all splitless injections. The injector and detector temperatures were set at 250 °C and 300 °C, respectively. The carrier gas helium and ECD makeup gas nitrogen were of ultra-high purity, respectively.

RESULTS AND DISCUSSION

The GC-MS method allowed us to detect the unknown pollutants in the liver extracts of the deceased wild animals. DDE and eleven PCBs (Table 1) were thus identified.

Usually, the polychlorinated hydrocarbons' extraction from animal tissues requires high solvent quantity and long extraction time [17-20]. Other techniques, like Matrix Solid Phase Dispersion (MSPD) overcomes many of these disadvantages [6, 21]. Recently, SFE offered us an alternative extraction that, minimizing time and amount of solvents, permitted adequate accuracy required to pollutants' residue determination like PCBs [15] and p,p'-DDE. In the present work, extracts obtained from chicken liver were analyzed by GC-ECD to determine the pollutants content and the choice of ECD detector for the quantification was suggested from its selectivity towards the target compounds. The effectiveness of the cleanup procedure allowed to obtain chromatographic analysis of liver samples sufficiently free of interfering extraneous peaks, in extracts of blank as well as in spiked samples (Fig. 1).

The calibration curves, each plotted with six points (concentration range 9 µg ml^{-1} – 0.014 µg ml^{-1}), were linear, and the relative equations are reported in Table 4, where y was the ratio of the peak areas pollutant to I.S. and x was the ratio of their concentrations. The linear-range experiments provided the necessary information to estimate the limits of detection (LODs) (Table 4), based on the lowest detectable peak that had S/N=3.

Recovery values of the method were studied by spiking liver samples at six (PCBs) and eight (p,p'-DDE) fortification levels with isooctane standard solutions of analytes and analyzing six replicates (Table 3).
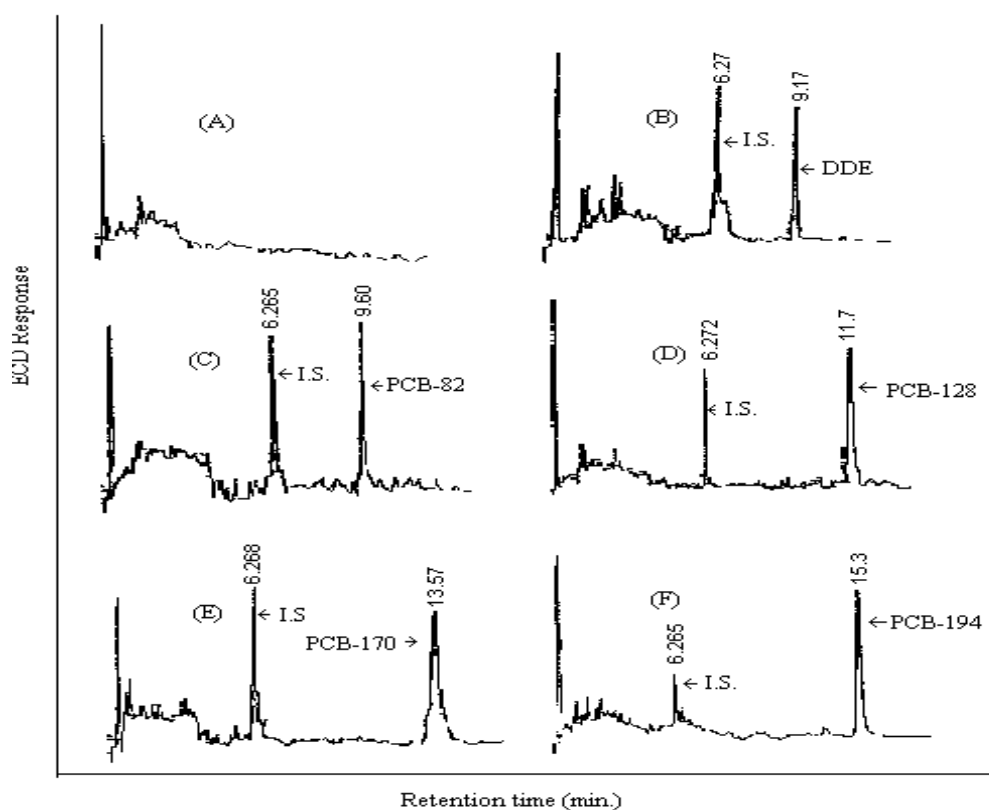
FIGURE 1 - GC-ECD chromatograms of extracts from chicken liver fortified with:
(A) blanc,(B) p,p¹-DDE, (C) PCB-82, (D) PCB-128, (E) PCB-170, (F) PCB-194.

TABLE 4
Equations of standard curves, regressional coefficients (r) and detection limits (LODs).

Pollutant	Equations	r	LODs (ng ml^{-1})
p,p¹-DDE	y = 0.005 (±0.0033) + 0.048 (±0.002)x	0.9980	3
PCB-82	y = −0.0015 (±0.0009) + 0.074 (±0.001)x	0.9997	8
PCB-128	y = 0.005 (±0.002) + 0.026 (±0.001)x	0.9983	8
PCB-170	y = 0.0042 (±0.0018) + 0.021 (±0.001)x	0.9974	10
PCB-194	y = 0.0098 (±0.0020) + 0.025 (±0.001)x	0.9980	9

TABLE 5
Regression line of "added" and "found" and relative recoveries.

Pollutant	Regression line and regressional coefficients (r)		Recovery %
PCB-82	y = −0.0527 + 0.8544x,	r = 0.9994	85.44
PCB-128	y = 0.059 + 0.804x,	r = 0.9978	80.40
PCB-170	y = 0.052 + 0.7576x,	r = 0.9990	75.76
PCB-194	y = −0.038 + 0.8047x,	r = 0.9995	80.47
p,p¹-DDE	y = −0.021 + 0.8864x,	r= 0.9998	88.64

TABLE 6
p,p' DDE and PCBs concentrations ($\mu g \ g^{-1}$) in liver of 51 sample of dying species.

Samples	p,p^1-DDE	PCBs-5-Cl	PCBs-6-Cl	PCBs-7-Cl	PCBs-8-Cl
1. Turtledove	—	—	—	—	—
2. Civet	0.4841	0.7070	1.8309	0.9986	—
3. Buzzard	0.0355	—	—	—	—
4. Kestrel	—	—	2.3760	2.0000	—
5. Barn owl	0.2373	—	1.2118	0.4588	0.2033
6. Hoopoe	0.1466	—	—	—	—
7. Starling	0.2541	—	—	—	—
8. Goshawk	3.9494	0.3504	5.9276	4.8469	1.2098
9. Golden eagle	0.6021	—	4.2830	2.6737	0.5856
10. Marten	0.0339	—	—	0.3204	—
11. Herring gull	0.0536	—	0.6091	0.2354	—
12. Horned owl	0.0706	—	—	—	—
13. Raven	0.0746	—	0.4136	0.1841	0.2855
14. Peregrin	0.4296	0.2685	2.9427	1.3246	—
15. Hare	—	—	—	—	—
16. Stone-Curlew	0.0317	—	0.2730	—	—
17. Horned owl	—	—	—	—	—
18. Horned owl	0.0949	—	—	—	—
19. Mouflon	0.0468	—	—	—	—
20. Golden eagle	0.3116	—	3.0964	1.7035	—
21. Egret	0.0405	—	—	—	—
22. Gull	0.0850	—	1.3461	1.6558	0.4071
23. Duck owl	1.5350	—	2.1659	1.5167	0.4136
24. Duck owl	0.3388	—	0.4186	—	—
25. Duck owl	0.0601	—	—	—	—
26. Sparrow-Hawk	0.3662	—	1.4392	0.4021	—
27. Fallow deer	—	—	—	—	—
28. Deer	—	—	—	—	—
29. Sea crow Cormorant	0.6396	0.5767	4.7829	1.3114	0.1749
30. Buzzard	—	—	—	—	—
31. Deer	0.0464	—	—	—	—
32. Flamingo	0.0476	—	1.4606	0.7135	0.4662
33. Raven	0.0902	—	0.8195	0.9791	0.3151
34. Buzzard	0.1406	—	2.8217	2.1564	0.5217
35. Hawk	0.5077	0.8765	8.0724	7.4872	1.9218
36.Griffon	0.1367	—	0.6080	0.1438	—
37. Gull	2.1010	4.1567	66.52	27.35	2.6560
38. Bittern	0.3062	—	0.4360	—	—
39. Heron	1.2055	2.9293	7.7217	1.6752	0.3832
40. Mouflon	—	—	—	—	—
41. Partridge	—	—	—	—	—
42. Kestrel	0.0838	—	0.6866	0.2585	—
43. Mouflon	—	—	—	—	—
44. Buzzard	—	—	0.7910	0.4680	—
45. Duck hawk	0.1652	0.2129	1.1238	0.3474	—
46. Crow	0.1339	—	0.7221	0.1865	—
47. Kestrel	—	—	—	—	—
48. Herring gull	1.0820	0.2109	2.3225	0.9379	—
49. Herring gull	0.1397	0.0372	2.4157	1.0737	—
50. Herring gull	0.0879	—	0.5885	0.2556	—
51. Herring gull	0.0497	—	—	0.1003	—

The concentration ranged from 10 to 0.05 µg ml^{-1} for PCBs and from 10 to 0.005 µg ml^{-1} for p,p^1-DDE. Least-squares and regression analysis of the data presented in Table 2 show that the relationships between "added" and "found" were adequately described by the linear regressions reported in Table 5. Therefore, for each analyte, the slope of regression line (Table 5) could be used as an estimate of recovery.

The present method provided us the necessary means for the quantification of PCBs and p,p^1-DDE residues in animal livers. Table 6 shows the results obtained after extraction and GC-ECD analysis of all 51 livers of the deceased specimens. p,p^1-DDE was present in 76.5% of the cases, while the PCBs, in 62.7% of the cases

This study may be considered as a starting point for further investigations and monitoring of the environment. Several authors reported that p,p^1-DDE concentrations higher than 10 µg ml^{-1} may affect reproduction in different mammalian species [22,23]. It is difficult to compare the present results with other studies on the subject, since little information is available and direct comparisons among the data are confounded by the different analytical techniques and methods of reporting and by the different species investigated.

CONCLUSIONS

In conclusion, the results of the present study show that the proposed SFE, SPE cleanup and GC-ECD method are an efficient and reliable means of quantitating p,p^1-DDE and PCBs in animal livers and would be useful for environmental pollution analysis. Although the use of DDT was legally banned from use in Sardinia, it continues to persist in the environment and bioaccumulate in the tissue of various species. No statistical differences were detected between sample sites.

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FEB

SYNTHESIS OF SEVERAL SINGLE C$_{10}$, C$_{11}$, AND C$_{12}$ CHLOROALKANES

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SUMMARY

Different chlorinated compounds with a chain length of C$_{10}$, C$_{11}$, and C$_{12}$ have been synthesized as single standards for the residue analysis of chlorinated paraffins. Two dichlorodecadienes, two tetrachlorodecenes, and two hexachlorodecanes could be isolated after stepwise chlorine addition to 1,5,9-decatriene. Through radicalic addition of CCl$_4$ to 1,5,9-decatriene, two tetrachloroundecadienes and two octachlorododecadienes were formed, which gave the corresponding octachloro-undecanes and decachlorododecanes after chlorine addition. Further chlorodecanes were obtained by HCl addition to dichlorodecadienes and tetrachlorodecenes. Elimination of one HCl from hexachlorodecanes yielded a mixture of several pentachlorodecene diastereoisomers and, after chlorination or hydrogenation of this mixture, a hexachlorodecene or a tetrachlorodecane respectively was isolated. Additionally, hydrogenation of tetrachlorodecenes and octachlorododecenes led also to the corresponding saturated chloro compounds. Furthermore, the number of compounds could be enlarged by the radical CCl$_4$ addition to 1,7-octadiene and 1,8-nonadiene, from which the corresponding octachlororodecane and octachloroundecane were obtained. The addition of one mole CCl$_4$ to 1,9-decadiene afforded a tetrachloroundecene, which reacted with chlorine to form hexachlorodecane.

KEYWORDS: chlorinated paraffins, synthesis, single chloro alkanes, quantification standards.

INTRODUCTION

Chlorinated paraffins (CPs) certainly represent the most complicated substance class of organochlorine compounds relevant to the environment. The extraordinary complexicity of the mixtures is due to the possibility of the formation of an extremely high number of isomers with the several n-alkane chains ranging from C$_{10}$ to C$_{30}$ combined with the variation in chlorination degree between 42-72 % [1,2]. Even the chlorination products of one n-alkane chain can be resolved only in several broad bands even by high resolution gas chromatography (HRGC) [3,4]. Therefore, industrially produced CPs are only grossly classified into three groups according to their chain length as short (C$_{10}$-C$_{13}$), middle (C$_{14}$-C$_{17}$) and long chain (C$_{20}$-C$_{30}$). Further analytical complications arise in view of differences between industrial products as well as by changes in composition through bio- and geoaccumulation or transformation of single components after application and dispersion resulting in mixtures greatly differing from industrial products. This leads to extreme difficulties with the quantification of CPs in environmental samples [4]. Hence, polychloroalkanes with defined chlorine content are preferably used as standards instead of technical CP products for the analytical determination. But the mass spectrometrical peak patterns of standards and samples are not always similar [4-6] and measured concentrations may vary with the response of the standards used for the quantification, which, in turn, depends on the degree of chlorination [4]. Therefore, single substances at least for each alkane chain and chlorine number were even more useful. Some polychloro alkanes with chain lengths of C$_{10}$, C$_{11}$, C$_{12}$ prepared by addition of molecular chlorine to the corresponding alkenes were recently used for studying the environmental behaviour of CPs but without purification and any separation of the products [7]. Nevertheless, only 5 different tetra- to hexachlorodecanes are commercially available [8]. The reasons for this may be not only the extremely high number of substances needed for a correct quantification of all possible mixtures, but also the problems with the synthesis of defined single isomers by the standard procedures of chlorination of alkanes or alkenes. To reduce the immense labor, we selected some structures of different chain length and chlorination grade, which promised the production with less trouble, and synthesized these componds by different chlorination methods and hydrogenation (Scheme 1).
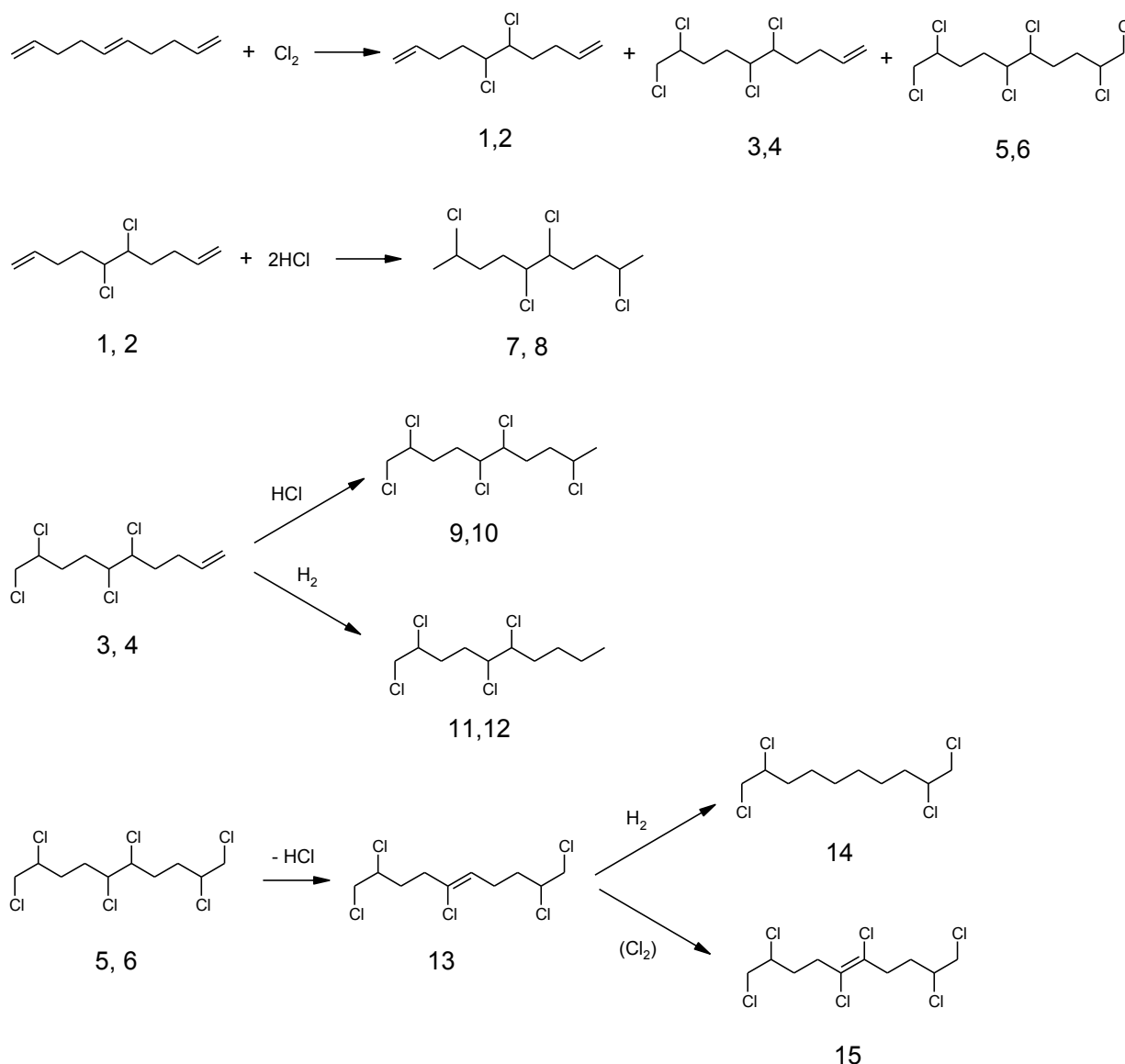
SCHEME 1 - Chlorination of 1,5,9-decatriene.

MATERIALS AND METHODS

1,5,9-Decatriene, 1,9-decadiene and 1,8-nonadiene were purchased from Aldrich (Steinheim, FRG). Petroleum ether (bp 40-65 °C) was distilled over a 1 m column. The other organic solvents with purity grade of analysis, fuming hydrochloric acid (37 %), palladium on activated carbon (10 % Pd), 1,7-octadiene, activated carbon for GC (35-50 mesh ASTM), 2,2'-azobis(2-methypropionitrile) (AIBN) and silica gel 60 (70-230 mesh) were obtained from Merck (Darmstadt, FRG). Stannous tetrachloride, p.a was from Fluka (Buchs, CH). Chlorine (purity of 99.9 %) was purchased from Messer-Griesheim (Germany).

The gel permeation chromatographic (GPC) system consisted of a quarternary pump, model 1050 from Hewlet Packard, a glass column (i.d. 20 mm) filled with 55 g Bio-Beads S-X3 (200-400 mesh) from Bio-Rad, and an UV-VIS detector (Merck Hitachi L-2400) were used. The flow rate was 3 ml/min (hexane/dichloromethane, 1:1).

The GC/MS measurements were performed with an HP 5970 MSD equipment. The column used was a DB5, 30 m, i.d. 0.25 mm, film thickness 0.25 µm. The GC temperature program was: 100 °C for 3 min, then at 5 °C/min to 280 °C and held for 15 min.

Synthesis

The compounds synthesized together with their retention times and mass spectrometrical fragment ions are listed in Table 1. All compounds isolated with one exception consist of several diastereomers which could not be separated further by column chromatography under the experimental conditions used. ^{1}H NMR and ^{13}C NMR data will be published elsewhere [9].

Compounds with the same designation but with different numbers, represent diastereomer mixtures obtained either from different starting substances (e.g 1→7, 2→8) or via separation by column chromatography (e.g decatriene →1 and 2).

Chlorination of 1,5,9-decatriene

1.75 g chlorine dissolved in 100 ml of CCl_4 was slowly dropped into an intensively magnetically stirred solution of 2.5 g 1,5,9-decatriene in 50 ml of CCl_4 at 0 °C. Another reaction charge was carried out under the same reaction conditions with 2.5 g 1,5,9-decatriene and 3.5 g chlorine. After completion of the reaction of chlorine, the two solutions were pooled and the solvent was rotary evaporated. The crude product was chromatographed on a column (100 cm × 5.5 cm i.d.) of silica gel with petroleum ether as eluent. The compounds 1-6 were eluted successively. If necessary, the chromatography was repeated to reach a purity of more than 95 %.

Synthesis of the compounds 16-19

15 g 1,5,9-decatriene were refluxed in 150 ml CCl_4 for 6 days, adding 0.5 g AIBN every day during refluxing. After that, the reaction solution was treated with 100 ml of conc. sulphuric acid. The organic phase was separated, washed with water, and rotary evaporated. The residue was dissolved in 50 ml petroleum ether and fractionated over a short silica gel column (50 cm × 5 cm) to separate 16 and 17 from 18 and 19. Thereafter, both compound mixtures were chromatographed with petroleum ether again on silica gel column (100 cm × 2.6 cm), whereas for the separation of 18 from 19 silica gel was activated before use at 150 °C for 24 hours.

Synthesis of the compounds 21, 23-25, and 27

The synthesis of compounds 21 and 27 (from 1,9-decadiene), 23 (from 1,7-octadiene), and 24 and 25 (from 1,8-nonadiene) was analogous with the above-described. Separation of diasteomers could not be achieved under the experimental conditions used. The products were purified on a silica gel column (100 cm x 2.6 cm) with petroleum ether.

Chlorination of the compounds 16-19, 24 and 27

300 mg of the compound was dissolved in 25 ml of CCl_4, to which 250 mg chlorine in 5 ml CCl_4 was added under exclusion of light. From 16 and 17, first hexachloroundecenes were formed, which were not isolated and the reaction was continued to octachloroundecane 20.

Chlorination of 18 and 19 yielded 22, and that of 24 the product 26. By the same method, compound 28 was obtained from 27. The purification of all end products was carried out on silica gel column with petroleum ether as described above.

Formation of 13

3 g of a mixture of 5 and 6 was mixed with 10 g of active carbon in a 250 ml round-bottom flask and under heating, in an oil bath slowly rotated at rotary evaporator at 160 °C for about 2 days. The reaction was finished when 13 reached a gas chromatographical concentration of about 65 %. Soxhlet extraction was carried out with petroleum ether. 13 was isolated with a purity of more than 99 % after silica gel column chromatography (100 cm × 2.6 cm) with petroleum ether.

Synthesis of the compounds 7-10

0.5 g of 1 (or 2-4) and 100 µl $SnCl_4$ were dissolved in 15 ml of water-free dioxane in a 20 ml headspace vial. HCl gas generated from conc. hydrocloric acid by heating in a round-bottom flask and dried over $CaCl_2$ was introduced into the dioxane solution up to saturation. After this, the sealed vial was kept in an oven at 60 °C for about two days. The course of reaction was followed gas chromatographically by injection of samples taken every 16 hours with a GC syringe. The products were extracted from dioxane with petroleum ether after the addition of water. For the purification a silica gel column (100 × 2.6 cm) with petroleum ether as eluent was used.

Hydrogenation of the compounds 3, 4,13, 18, and 19

0.5 g of 3 (or 4, 13, 18, 19) was dissolved in 50 ml of ethylacetate in a round-bottom flask with two necks and 50 mg Pd/on activated carbon was added. Hydrogenation of the magnetically stirred sample was carried out under about 5 psi H_2 of overpressure. After completion of the reaction, the solution was filtered, the solvent rotary evaporated and redissolved in petroleum ether. Contrary to 3, 4 and 13, the hydrogenation products of 18 and 19 are identical, and could be isolated only in about 50 % purity after threefold chromatography on silica gel column (100 × 2.6 cm). Therefore, these samples were further chromatographed on a GPC column with hexane:dichloromethane (1:1). Finally, a purification again on silica gel was necessary to obtain the product with a purity of more than 90 %.

RESULTS AND DISCUSSION

Chlorine addition to 1,5,9-decatriene

The 5,6-dichloro-1,9-decadienes (1 and 2), 5,6,9,10-tetrachloro-1-decenes (3 and 4) and 1,2,5,6,9,10-hexachlorodecanes (5 and 6) were formed in dependence on the chlorine amount, besides small amounts of some by-products. Chlorine addition occurs first at the middle double

TABLE 1 - The list of the synthesized compounds, their retention times (as minutes) and relative abundance of some mass spectrometrical fragment ions.

No	chemical name	retention time	EI
1	5,6-dichloro-1,9-decadiene	9.90	206 (M^+, <0.1), 171 ($M-Cl^+$, 4.9), 135 ($M-Cl-HCl^+$, 20.8), 67 (100)
2	5,6-dichloro-1,9-decadiene	10.22	206 (M^+, 0,1), 171 ($M-Cl^+$, 5.5), 135 ($M-Cl-HCl^+$, 23.3), 67 (100)
3	5,6,9,10-tetrachloro-1-decene	21.05	276 (M^+, 0.5), 241 ($M-Cl^+$, 2.0), 240 ($M-HCl^+$, 2.8), 205 ($M-Cl-HCl^+$, 29.1), 67 (100)
4	5,6,9,10-tetrachloro-1-decene	21.27, 21.35	276 (M^+, 1.1), 241 ($M-Cl^+$, 2.4), 240 ($M-HCl^+$, 2.9), 205 ($M-Cl-HCl^+$, 32.0), 67 (100)
5	1,2,5,6,9,10-hexachlorodecane	30.98	346 (M^+, 0.5), 310 ($M-HCl^+$, 11.5), 275 ($M-Cl-HCl^+$, 29.1), 274 ($M-2HCl^+$, 10.1), 75 (100)
6	1,2,5,6,9,10-hexachlorodecane	31.1, 31.17	346 (M^+, 0.2), 310 ($M-HCl^+$, 5.3), 275 ($M-Cl-HCl^+$, 18.8), 274 ($M-2HCl^+$, 5.9), 75 (100)
7	2,5,6,9-tetrachlorodecane	21.4, 21.55, 21.66	278 (M^+, <0.1), 242 ($M-HCl^+$, 2.1), 206 ($M-2HCl^+$, 30.7), 103 (100)
8	2,5,6,9-tetrachlorodecane	21.53, 21.69, 21.85	278 (M^+, 0), 242 ($M-HCl^+$, 3.9), 206 ($M-2HCl^+$, 29.8), 103 (100)
9	1,2,5,6,9-pentachlorodecane	26.32, 26.45	312 (M^+, 0), 276 ($M-HCl^+$, 9.6), 241($M-Cl-HCl^+$, 17.1), 240 ($M-2HCl^+$, 22.7), 103 (100)
10	1,2,5,6,9-pentachlorodecane	26.42, 26.57, 26.66	312 (M^+, 0), 276 ($M-HCl^+$, 6.7), 241($M-Cl-HCl^+$, 13.5), 240 ($M-2HCl^+$, 19.2), 103 (100)
11	1,2,5,6-tetrachlorodecane	21.41	278 (M^+, 0), 242 ($M-HCl^+$, 4.2), 207 ($M-Cl-HCl^+$, 15.6), 206 ($M-2HCl^+$, 5.9), 69 (100)
12	1,2,5,6-tetrachlorodecane	21.60, 21.68	278 (M^+, 0), 242 ($M-HCl^+$, 3.1), 207 ($M-Cl-HCl^+$, 12.2), 206 ($M-2HCl^+$, 4.3), 69 (100)
13	1,2,5,9,10-pentachloro-5-decene	26.75	310 (M^+, 18.8), 275 ($M-Cl^+$, 13.0), 274 ($M-HCl^+$, 1.8), 239 ($M-Cl-HCl^+$, 19.3), 91 (100)
14	1,2,9,10-tetrachlorodecane	23.81	278 (M^+, 0), 242 ($M-HCl^+$, 0.1), 207 ($M-Cl-HCl^+$, 4.6), 206 ($M-2HCl^+$, 5.4), 55 (100)
15	1,2,5,6,9-hexachloro-5-decene	29.18	344 (M^+, 33.0), 309 ($M-Cl^+$, 9.8), 308 ($M-HCl^+$, 6.1), 273 ($M-Cl-HCl^+$, 30.0), 125 (100)
16	9,11,11,11-tetrachloro-1,5-undecadiene	19.51	288 (M^+, 0), 253 ($M-Cl^+$, 1.0), 217 ($M-Cl-HCl^+$, 1.0), 67 (100)
17	9,11,11,11-tetrachloro-1,5-undecadiene	19.73	288 (M^+, 0), 253 ($M-Cl^+$, 1.2), 217 ($M-Cl-HCl^+$, 1.4), 67 (100)
18	1,1,1,3,10,12,12,12-octachloro-6-dodecene	35.48	440 (M^+, 7.2), 405 ($M-Cl^+$, 0.7), 404 ($M-HCl^+$, 1.2), 369 ($M-Cl-HCl^+$, 7.4), 368 ($M-2HCl^+$, 1.0), 67 (100), 109 (99)
19	1,1,1,3,10,12,12,12-octachloro-6-dodecene	35.74	440 (M^+, 8.0), 405 ($M-Cl^+$, 0.5), 404 ($M-HCl^+$, 0.7), 369 ($M-Cl-HCl^+$, 7.2), 368 ($M-2HCl^+$, 0.9), 67 (97), 109 (100)
20	1,1,1,3,6,7,10,11-octachloroundecane	36.73, 36,83	428 (M^+, 0), 392 ($M-HCl^+$, 6.6), 357 ($M-Cl-HCl^+$, 14.4), 356 ($M-2HCl^+$, 8.0), 75 (100)
21	1,1,1,3,10,12,12,12-octachlorododecane	36.45	442(M^+, 8.0), 371 ($M-Cl-HCl^+$, 3.1), 370 ($M-2HCl^+$, 1.5), 335 ($M- Cl-2HCl^+$, 21.7), 299 ($M- Cl-3HCl^+$, 22.1), 109 (100)
22	1,1,1,3,6,7,10,12,12,12-decachlorododecane	44.71, 45.04, 45.37	510(M^+, 0), 474 ($M-HCl^+$, 3.2), 439 ($M-Cl-HCl^+$, 6.5), 438 ($M-2HCl^+$, 5.0), 403 ($M- Cl-2HCl^+$, 16.8), 109 (100)
23	1,1,1,3,8,10,10,10-octachlorodecane	32.47	414 (M^+, 0), 379 ($M-Cl^+$, 0.1), 378 ($M-HCl^+$, <0.1), 343 ($M-Cl-HCl^+$, 6.3), 342 ($M-2HCl^+$, 1.3), 109 (100)
24	8,10,10,10-tetrachloro-1-decen	17.76	276 (M^+, 0.1), 241 ($M-Cl^+$, 0.4), 240 ($M-HCl^+$, 0.4), 234 ($M-C_3H_6^+$, 1.5), 55 (100)
25	1,1,1,3,9,11,11,11-octachloroundecane	34.51	428 (M^+, 0), 357 ($M-Cl-HCl^+$, 4.0), 321 ($M- Cl-2HCl^+$, 15.7), 109 (100)
26	1,1,1,3,9,10-hexachlorodecane	28.33	346 (M^+, 0), 311 ($M-Cl^+$, 0.6), 310 ($M-HCl^+$, 0.4), 275 ($M-Cl-HCl^+$, 33.2), 274 ($M-2HCl^+$, 5.8), 55 (100)
27	9,11,11,11-tetrachloro-1-undecene	20.3	290 (M^+, 0), 255 ($M-Cl^+$, 0,2), 254 ($M-HCl^+$, 0.4), 219 ($M-Cl-HCl^+$, 2.7), 55 (100)
28	1,1,1,3,10,11-hexachloroundecane	30.49	360 (M^+, 0), 289 ($M-Cl-HCl^+$, 9.3), 288 ($M-2HCl^+$, 2.7), 253 ($M- Cl-2HCl^+$, 40.0), 55 (100)

bond of 1,5,9-decatriene. According to gas chromatographic separation, the compounds **1** and **2** are formed after the reaction of equiv. moles of chlorine and 1,5,9-decatriene. Some 5,6,9,10-tetrachloro-1-decenes are also formed before completion of reaction of 1,5,9-decatriene. After the addition of 2 eq. moles of chlorine, tetrachlorodecenes were the main products, as expected, besides some dichloro and hexachloro compounds. By addition of more chlorine, hexachlorodecanes were the only new products. But after that, when the reaction solution was refluxed with an excess of chlorine, several hepta to octachlorodecanes were formed, which could not be separated completely by HRGC. The isolation of hexachlorodecanes from the mixture was achieved by crystallisation from methanol. When hexane instead of methanol was used, **6** could be isolated in relatively pure form after several recrystallisations. Further purification was done by column chromatography. The compounds **1-6** could also be isolated from one mixture containing **1-6** by column chromatography, instead of carrying out the separation three times for di- tetra- and hexachlorodecanes. For that, a mixture of **1-6**, which contained all the components in about similar proportions, was needed. That could be obtained by pooling of two reaction mixtures. For that purpose, two chlorinations had to be carried out with about 1.3 and 2.6 moles eq of chlorine. The elution sequence of compounds on silica gel column was the same as in the gas chromatography. For the complete separation of isomer pairs (**1**:**2**, **3**:**4**, and **5**:**6**), column chromatography had to be carried out several times.

HCl elimination from the compunds 5 and 6,
and subsequent chlorine addition

First, bases such as KOH, NaOH and several amines were used. Because the products were rather complex by this way, catalytical elimination on activated charcoal was preferred. According to their gas chromatographical retention time, **5** and **6** led to the same products. The yields on Al_2O_3 were somewhat lower. The reaction was not continued up to complete decomposition of **5** and/or **6**, because even with longer reaction time the yields were not higher and, moreover further eliminations occurred and, therefore, the mixture would become more complex. In addition to **13**, other isomers in low proportions were formed. Even after several column chromatographical attempts, these isomers could not be isolated in pure form. Hydrogenation of **13** provided 1,2,9,10-tetrachlorodecane (**14**) and not as expected, 1,2,5,9,10-pentachlorodecane. Chlorine addition to **13** was a slow reaction process. As major components, a hexachlorodecene (**15**) and a heptachlorodecane were formed, besides several by-products. The heptachloro product could not be isolated in pure form by column chromatogrphy on silica gel. Both compounds are probably mixtures of diastereomers. The formation pathway of 14 could not be clarified.

HCl addition to the compounds 1, 2, 3 and 4

The addition of HCl does well in the dried solvents methanol, tetrahydofurane, dioxane and diethyl ether, but not alike in all solvents. Thus, an unidentified by-product is formed in diethyl ether up to 18 % detected by gas chromatography. In tetrahydofurane, the reaction was somewhat slower. In methanol and in dioxane the reaction occurs very similar. But, all HCl-addition reactions have been carried out in dioxane, because of reaction temperature of 60 °C.

Radical CCl₄ addition to 1,5,9- decatriene, 1,7-octadiene, 1,8-nonadiene and 1,9-decadiene

First, with N-chlorosuccinimide as radical promotor chlorination of 1,5,9-decatriene was attempted at allylic positions. That is a reaction, which does not always take place with every alkene. After a long refluxing time, some chlorinated compounds with chain length C11 and C12 were formed (Scheme 2). The products were two 9,11,11,11-tetrachloro-1,5-undecadienes (**16**,**17**) and two 1,3,3,3,10,12,12,12-octachloro-6-dodecenes (**18**,**19**). The presence of N-chlorosuccinimide had no significant effect on the addition reaction. The desired compounds mono, di, tri and perhaps 3,4,7,8-tetrachlorodecatrienes could not be detected in significant amounts. Further experiments for achieving this aim has not been carried out, but this procedure may be a possible method of synthesis for several differently substituted chlorinated decanes with a suitable chlorination agent for allylic position. The addition of CCl₄ to 1,5,9-decatriene required refluxing for several days. By the use of benzoyl peroxide instead of AIBN as radical promotor, the reaction was too slow. With time a lot of by products were formed, whereas a lot of them could be eliminated by H_2SO_4 treatment. The tetrachloroundecadienes could be separated from octachlorododecenes without any difficulty by column chromatography. The separation of isomers, on the other hand, was complicated, so that chromatography had to be carried out several times.

The addition of CCl₄ to 1,7-octadiene, 1,8-nonadiene or 1,9 decadiene afforded initially tetrachloro compounds and by further reaction octachloro compounds (Scheme 2). The synthesis of **21** from 1,9-decadiene should be preferred to that from 1,5,9-decatriene via **18** or **19** because of high purity of product obtained. CCl₄ addition to olefins containing terminal double bonds has been well studied by Kharasch and co-workers [10]. They found the radicalic addition of CCl₄ to olefinic compounds could be initiated also by ultaviolett light.

Hydrogenation of the compounds 3, 4, 13, 18, and 19.

All hydrogen addition reactions have been carried out with Pd/on activated carbon as catalyst in ethyl acetate. The hydrogenation of *18* and **19** was somewhat complex, because several by-products with low proportions were formed. Removing of by-products was rather difficult. In spite of repeated chromatography on silica gel, the purity could not be raised clearly above 50 %. Therefore, the purification has been continued with GPC on Bio-Beads gels, so that a purity of about 80 % could be achieved.
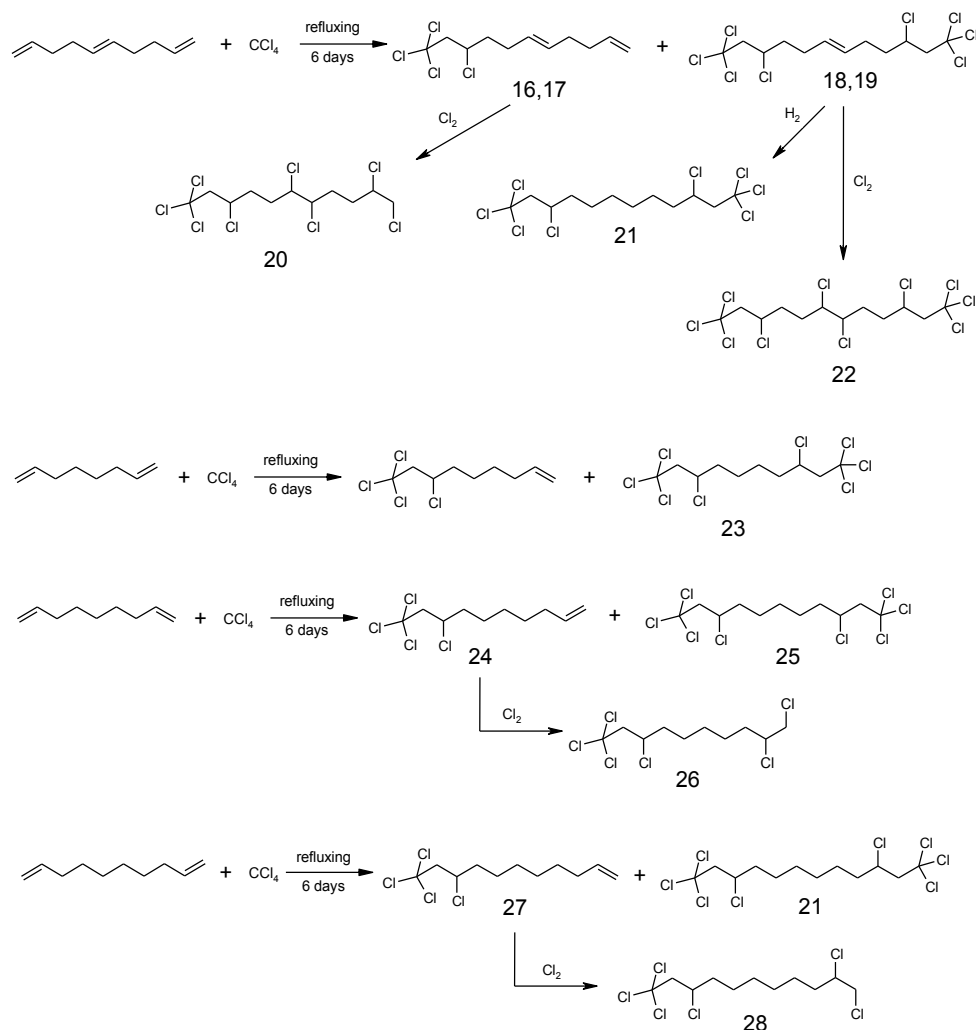
SCHEME 2 - Synthesis of the compounds *16-28*.

By subsequent fractioniation on silica gel the both compounds could be obtained with about 93 % purity. According to gas chromatographical and also ^{1}H-NMR studies the hydrogenation products of **18** and **19** are identical.

**Cl$_2$-addition to the compounds 16, 17, 18 and 19**

By the reaction of **16**+**17** with chlorine at least three hexachloroundecenes were formed at first, which were not completely separable even by HRGC. With further chlorine addition, the hexachloroundecenes reacted to at least four octachloroundecanes. A separation of isomers could not be achieved by silica gel column chromatography.

The chlorine additions to **18** or **19** yielded at least three octachlorododecanes, which seemed to be identical according to the gas chromatogramms. Therefore, both mixtures were combined and the impurities removed on silica gel. Although three peaks could be registered clearly in the gas

chromatogramms, the real number of isomers should be higher on ground of forming of peak shoulders.

Whether all the synthetised compounds are really present in the commercial CP mixtures ist not known. On the other hand, it can not be expected for the near future that single components will be isolated from these complex mixtures, because even HRGC is not sufficient for an acceptable separation. However this circumstance should not prevent the use of compounds since they represent definied substances. Probably, the presented compounds suit better for compound-related studies of the metabolic and environmental fate of CPs than for analytical purposes due to the lack of compounds with 7, 9, and 10 chlorine atoms. For the first aim mentioned before particularly chlorodecanes come into question, as several compounds are now available with different chlorine substitutions. Then, more than the half of synthesized compounds have a C10 chain.
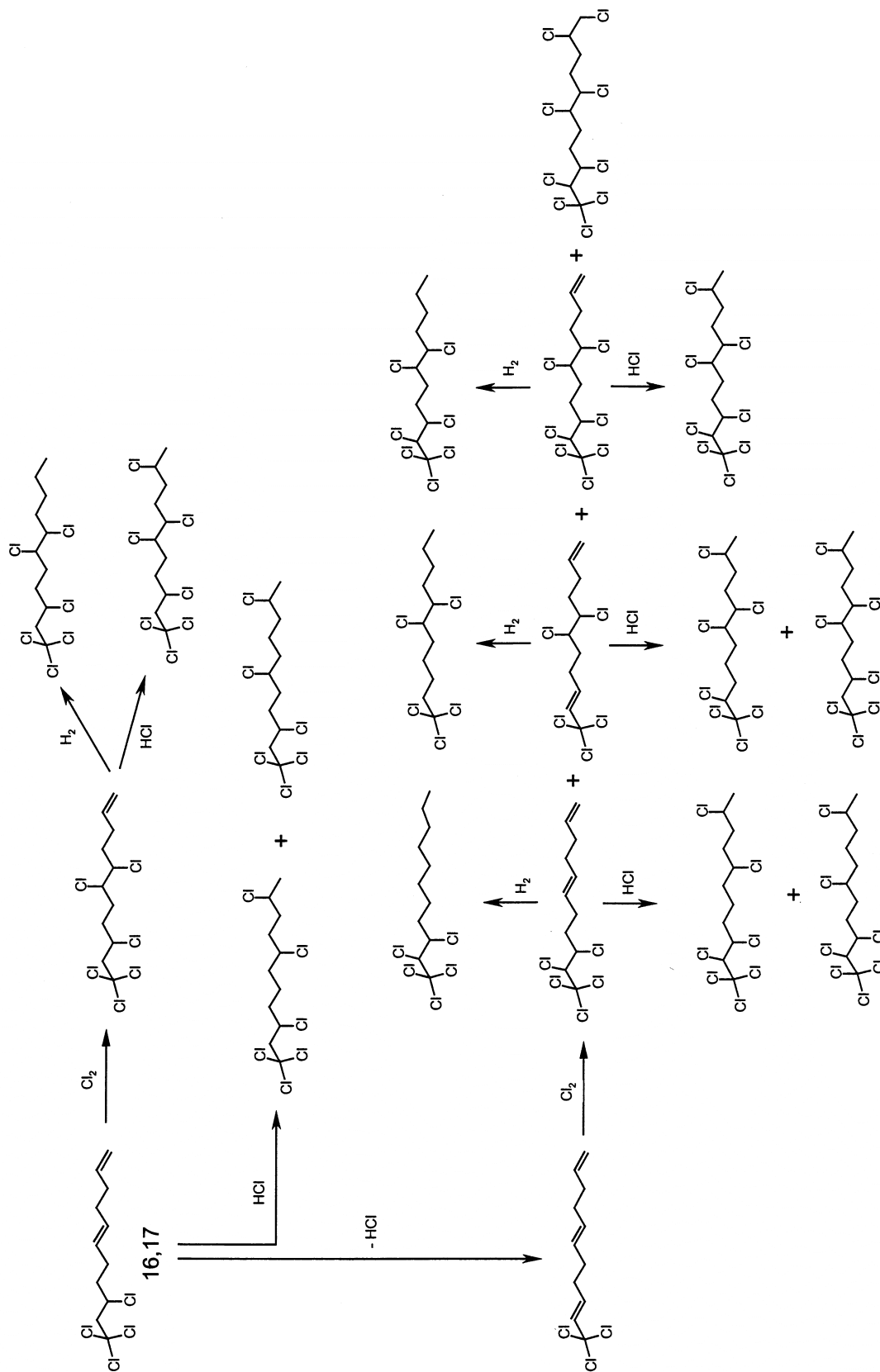
SCHEME 3 - Probable synthesis pathways of several chloroundecanes from *16* and *17* as starting compounds.

CONCLUSIONS

In a recent study we performed XR rontgen analysis for the compounds **5** and **6** [11]. The gas chromatographical peak of **6** appears as double in the chromatogramms, that indicates a mixtures of racemats. Contrarily a crystal of **5** analysed consisted of only one enantiomer with the structure RS, RR, RR, RS. Although resolution of enantiomers by crystallisation is rather rare, it does occur occasionally [12]. So this must be one of these seldom cases, because compounds **5** and **6** consist of three and four isomers, respectively, according to NMR Studies [9].

The number of isomers synthesized can be enlarged from the compounds presented by HCl elimination and subsequent chlorine addition. For example, 1,10-dichloro-2,4,6,8-decatetraene should be formed from 1,2,5,6,9,10-hexachlorodecane after elimination of four HCl-groups. After that, by step-wise chlorine addition and, if necessary, H_2 addition, compounds with chlorine number up to 10 can be isolated. It must be considered that compounds with multiple double bonds can react before chlorine addition. By HCl-addition to **16**,**17**, **18** and **19** compounds with chlorine number up to 9 should be obtained (Scheme 3). But if first HCl will be eliminated and then chlorine added, the palette of isomers or new compounds will be greater.

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TRACE ELEMENT BIOMONITORING BY NEEDLES OF

Pinus brutia TEN. FROM WESTERN ANATOLIA, TURKEY

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SUMMARY

The needles of *Pinus brutia* were used as biomonitor in Western Anatolia to investigate the levels of the trace elements (Pb, Cu, Fe, Ni and Cu) by flame atomic absorption spectrometry. The samples were collected at both reference and contaminated locations, where iron was found as the priority element. Levels of Cd were similar to those in unpolluted areas, whereas high values were found for Pb, Cr, Cu, Ni and, especially, Fe.

KEYWORDS:
Trace element, biomonitoring, pollution, *Pinus brutia*, flame atomic absorption spectrometry (FAAS), Western Anatolia.

INTRODUCTION

Metals, such as Cd, Pb, Cr, Fe, Ni and Cu, are essential components of many alloys, wires, tires and many industrial processes, and could be released into the roadside soil and incorporated in plants as a result of mechanical abrasion and wear. Analyses of plants revealed that they contain elevated levels of these heavy metals [1, 2]. Toxicities of some heavy metals to man and animals are well known [3]. Of all the toxic metals in the environment, lead is by far the one of most concern; it affects thousands of people annually, especially children in urban areas [4].

Because of the broad geographical distribution, sedentariness and easy sampling as well as the pollutants' impact perceptivity, plants are widely used for the monitoring of trace elements. Nevertheless, plants have different sensitivity according not only to the type of pollutant contaminant, but also their growth period [5]. It is, therefore, difficult to calibrate sampling procedures to achieve a very small subsample with the average composition of the plant population under study [6].

Plant species have been increasingly used as bioaccumulators of trace elements [7]. Among plants, trees are rather toxitolerant and their leaves are the principal interceptor of airborne metals in forest ecosystems. However, element uptake via the roots obscure the interpretation of analytical data in terms of airborne deposition [8]. For that reason, leaves of higher plants have been used for the biomonitoring of trace elements since 1950's [9].

Aksoy et al. [10, 11] studied the leaves of *Elaegnus angustifolia* and *Robinia pseudo-acacia* as biomonitors of heavy metal pollution in Kayseri, Turkey. They determined Pb, Cd, Cu and Zn in the leaves collected from several sites in Kayseri. The metal concentrations were high in the leaves.

In this study, the needles of *Pinus brutia* Ten. (red pine) (Pinaceae), a typical forest vegetation in the region of Western Anatolia, have been used for the biomonitoring of Cd, Pb, Cr, Fe, Ni and Cu.

MATERIALS AND METHODS

The study was carried out in western region of Turkey between July and August 2000. The areas of the study (Figure 1) included industrial, roadside, rural and suburban (about 100.000 km^2 area). The mountains of Akdağ, Bozdağ, Murat Dağı and Kazdağı were selected as control sites. The samples were collected 2 m away from roads and at about 1000 m height of the mountains. The trees used for sampling were of the same age (about 40 years) and healthy. The needles were carefully removed with a stainless steel pen knife at an average height of about 3 m above the ground. Five needles from each directions (west, east, south and north) of the trees were collected. The middle part of the needles (about 3 years old) was used for sampling. The collected samples were dried in the shadow for one month. The dried material was ground and sieved (0.2 mm).

FIGURE 1 - Map of study area.

Reagents and Instrumentation: All chemicals used were of analytical reagent grade unless otherwise specified. Triply distilled water was used throughout the experiments. Working metal standard solutions were prepared just before use by diluting the stock standard solution with water.

Determination of the metals was performed with Perkin Elmer AAnalyst 700 model flame atomic absorption spectrometer equipped with deuterium background correction, hollow cathode lamps (HCL) and acetylene burner. The absorption measurements of the metals were performed under the conditions recommended by the manufacturer. A Cole-Parmer microfiltration apparatus with membrane filter (0.45 µm pore size manufactured by Micro Filtration Systems, MFS) was used for the filtration of the aqueous phase before metal determination.

Wet Digestion Procedure: The method used for plant digestion was according to that described by Perkin Elmer Corporation [12]. 1 g of ground dried plant sample was put in a 100-ml beaker. Ten ml of concentrated HNO_3 was added and allowed to stand overnight covered with a watch glass. Then the beaker was heated carefully on a hot plate until the production of red NO_2 fumes has ceased. The solution was cooled and 2 ml of $HClO_4$ was added. The beaker was heated again until a small volume remained. After that the solution was filtered through a membrane filter (pore size 0.45 µm), transferred to a 25-ml flask and diluted with triply distilled water. Then the solution was aspirated into an air-acetylene flame and the metals were determined by flame atomic absorption spectrometry (FAAS). Three replicates were carried out for absorbance measurement for each experiment. The samples were spiked with a known amount of analytes to test the accuracy of the method. All samples were analyzed as soon as possible after preparation. Each experiment was repeated three times.

RESULTS AND DISCUSSION

The levels of Cd, Pb, Cr, Fe, Ni and Cu (µg g^{-1} dry weight) in the leaves of *Pinus brutia* collected from the seven aforementioned sampling stations in Western Anatolia, Turkey, are given in Table 1. Contents of Cd, Pb, Cr, Fe, Ni, and Cu (µg g^{-1} dry weight) ranged from 0.37 to 0.54, 2.8 to 12.1, 0.83 to 12.12, 93.13 to 595.5, 4.1 to 10.4 and 3.1 to 10.1, respectively, in contaminated sites. Contents of those metals (µg g^{-1} dry weight) were 0.43, 1.4, 0.78, 57.28, 0.88 and 1.1, respectively, in control sites.

Cadmium is one of the elements of greatest environmental concern. The concentration of this element was found decidedly low in the whole study area, with the values closer to those found in needles from the control sites. Cadmium contamination is low in the whole study area. As far as lead, chromium, iron, nickel and copper are concerned, the values measured in the vicinity of contamination sources were considerably higher than those found in the control sites. Iron is one of the principal element in the Earth crust. The high values of iron in this study may be due to the uptake through the root system. The comparison of iron levels between the contaminated and control sites shows that higher levels in the contaminated sites. It could be accepted as the iron contamination in the environment. The high heavy metal content in roadside and urban plant samples is mostly due to the density of the traffic, which is considered as one of major sources of heavy metal contamination, because unleaded gasoline is expensive and drivers prefer leaded gasoline. This causes high Pb pollution alongside the roads in Turkey. There is a crowded motor traffic consisting largely of heavily-laden trucks in the study area. This is also a major source of heavy metal pollution in the study area.

TABLE 1
Trace element concentrations (µg g^{-1}, dry weight) and statistical
evaluation in needles of *Pinus brutia* from Western Anatolia, Turkey.

Site	N	Cd	Pb	Cr	Fe	Ni	Cu
				Element concentration*, µg g^{-1} dry weight			
Industry	8	0.37 ± 0.2	8.3 ± 0.8	12.12 ±1.3	595.5 ± 43.3	5.9 ± 1.2	10.1 ± 1.8
Roadsite	6	0.45 ± 0.4	12.1 ± 1.1	6.4 ± 0.4	500.9 ± 36.8	4.1 ± 0.3	5.3 ± 0.3
Rural	6	0.54 ± 0.2	3.6 ± 0.9	9.9 ± 1.5	236.4 ± 13.7	10.4 ± 0.9	3.1 ± 0.3
Suburban	6	0.42 ± 0.2	2.8 ± 0.6	0.83 ± 0.1	93.13 ± 9.7	6.29 ± 1.0	3.91 ± 0.4
Control	4	0.43 ± 0.1	1.4 ± 0.3	0.78 ± 0.2	57.28 ± 4.6	0.88 ± 0.1	1.1 ± 0.2

*The mean of N determinations

The results found in this study may agree with the findings of other researchers [13-22]. All of them found that the sources of heavy metal contents in atmosphere, soils, plants and mosses are mainly due to traffic and industry.

From the results it can be concluded that the needles of *Pinus brutia* could be successfully used as suitable biomonitor for metal pollution in the environment.

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