Poster Session 1 - Ecotoxicology and Ecotoxicology & Environmental Health

P 1

A multibiomarker approach to assess environmental pollution in the Lagoon of Venice (Italy). I: the clam Tapes philippinarum as sentinel species

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The aim of the present study was to assess effects of environmental contaminants using a multibiomarker approach in the clam Tapes philippinarum from the Lagoon of Venice. Bivalves were collected in June 2007 in 8 sites of the Lagoon (Campalto, Marghera, Palude del Monte, Valle di Brenta, Cà Roman, San Servolo, Fusina and Canale Dese), differently influenced by both anthropogenic impact and natural conditions. The following biomarkers were chosen: total haemocyte count (THC) and lysozyme-like activity in cell-free haemolymph as immunomarkers, acetylcholinesterase (AChE) activity in gills as a biomarker of exposure to neurotoxic compounds, vitellogenin (Vg)-like protein levels in both digestive gland and cell-free haemolymph as a biomarker of exposure to estrogenic compounds, and survival-in-air widely used to evaluate general stress conditions in clams. The results showed that clams sampled at Marghera and Fusina, near the mainland industrial area of Porto Marghera, had significantly reduced THC values with respect to those of animals from the other sites. Conversely, significantly higher THC was observed in clams from Valle di Brenta and Cà Roman, the former close to the inner border of the Lagoon, influenced by wastewater from agricultural areas, the latter near the lagoon inlet, where intense fishing and passage of ships occur. Significantly increased lysozyme-like activity was recorded in cell-free haemolymph of clams collected at Campalto, influenced by effluents from a sewage treatment plant, Marghera and Cà Roman, suggesting that destabilisation of cell membranes occurred in haemocytes. Lower AChE activity was recorded in gills of clams collected at Fusina and San Servolo, indicating a probable exposure of animals to neurotoxic compounds, which originate from both agricultural and industrial effluent discharge and the urban area of the city of Venice, San Servolo being particularly influenced by municipal effluents from the city. Interestingly, higher Vg-like protein levels were observed in digestive gland and haemolymph of both male and female clams from Campalto, Marghera, Cà Roman, Valle di Brenta and San Servolo. Vg induction is generally recognised as a biomarker of exposure to estrogenic compounds. However, in the light of recent findings concerning involvement of Vg in immune responses (Zhang et al., 2005, Fish Shellfish Immunol., 19: 93-95), evaluation of Vg induction as potential immunomarker in laboratory and field studies is suggested. Significant variations in clam resistance to air exposure were also shown. LT50 values fell from 13 days in clams from Canale Dese, far from urban and industrial sources of pollution, to 6 days in animals collected at Marghera and Fusina, and to 5 days in bivalves from Cà Roman and San Servolo. On the basis of the biomarkers analysed, a homogeneous spatial distribution of contaminants in the Lagoon can be hypothesised, and no hot spot was highlighted. However, clams from less impacted sites in the northern Lagoon, such as Canale Dese and Palude del Monte (the latter used as a licensed area for clam culture), exhibited an overall better condition. For a more exhaustive evaluation of the results obtained, influence of both exogenous (i.e., water temperature and salinity, food availability) and endogenous factors (i.e., reproductive cycle) on functional responses investigated have to be taken into proper account.

P 2

Two dimensional hetero correlation analysis and statistical total correlation analysis applied to optical spectroscopic techniques used in ecotoxicological studies

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In this paper the algal culture of Dunaniella tertiolecta, a widely used marker of environmental quality were submitted to the action of several pollutants and then analyzed by visible (VIS) and infrared (FTIR) spectroscopic techniques and then elaborated by powerful mathematic approaches such as two-dimensional hetero correlation analysis (2DHCA) and statistical total correlation analysis (STCA). These approaches give further and precious information for obtaining specific differentiations in the toxic action of each pollutant allowing the identification of specific structural damages into the living cells and the identification of stress factors such as the increased production of chlorophyll a in presence of some specific pollutants. This information cannot be retrieved by the conventional approach when the solutions of algal cultures are examined by an optical microscope or by an automatic counter particle for the determination of the toxic effect of a pollutant. The results suggest that our proposed approach could be a useful complementary tool to the traditional ecotoxicological measurements of environmental quality.

P 3

Copper effects on cytokines and steroidogenic acute regulatory (StAR) gene expression in Sparus aurata head kidney cells

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Aims: The teleost head kidney is a complex tissue, composed by hematopoietic, lymphoid cells and immunocytes (macrophages, lymphocytes), as well as endocrine cells, namely chromaffin and corticosteroidogenic cells, that secrete catecholamines and corticosteroids, respectively. Because of its anatomical organization and rich blood supply, the head kidney is an important target for xenobiotics, including metals. Previous in vivo and in vitro studies concerning head kidney functions revealed that metals induce changes in cortisol secretion; however, the mechanisms that cause these changes are not yet well understood. Similarly, the information about metal effects on fish immune function is still scarce. One cannot disregard that the immune and neuroendocrine systems are closely linked and that bi-directional communication between these two systems is essential for the maintenance of homeostatic functions (Harris and Bird, 2000). Thus, the effects of metals on immune function can be either direct or mediated through the activation of hormonal axes.

There is strong evidence that the disruption of the steroidogenic pathways is mediated by effects on the steroidogenic acute regulatory protein (StAR), pointing out its important role as regulator of corticosteroidogenesis in fish (Arukwe, 2008). Despite these findings, there is little information about StAR gene expression and regulation in fish, namely after metals exposure. Previous studies have demonstrated that stress reduce lymphocyte proliferation and impairs antibody responses (Prophete et al., 2006), ultimately leading to an enhanced susceptibility to microbial agents of the water environment. It is also known that many cytokines interact with the cortisol production pathways. In particular, interleukins-1 and -6 (IL-1β, IL-6) as well as tumor necrosis factor (TNF-α) are considered key molecules in this communication between immune and neuroendocrine system (Engelsma et al., 2002).

Copper (Cu) is commonly found in aquatic environment in potentially hazardous levels to fish. Though essential for some physiological processes, Cu may become toxic at abnormally high cellular concentrations. Therefore, the present study was undertaken to assess the in vitro effects of Cu on the expression of StAR and the pro-inflammatory cytokines IL-1β, IL-6 and TNF-α in Sparus aurata (seabream) head kidney cells. Furthermore, possible immune-endocrine interactions are considered.

Methods: Head kidney preparations and in vitro assays

The experiments were carried out in seabream (100 g) from a fish farm (Vilanova, Barcelona) and maintained in a closed seawater (37‰ salinity) flow circuit. Seabream head kidney cells were isolated and cultured as previously described by Castillo et al. (2008) and after that incubated with medium with or without Cu (1 or 100 μM) for 1 h (n=4).

Analysis of gene expression in head kidney cells by quantitative Real-Time PCR

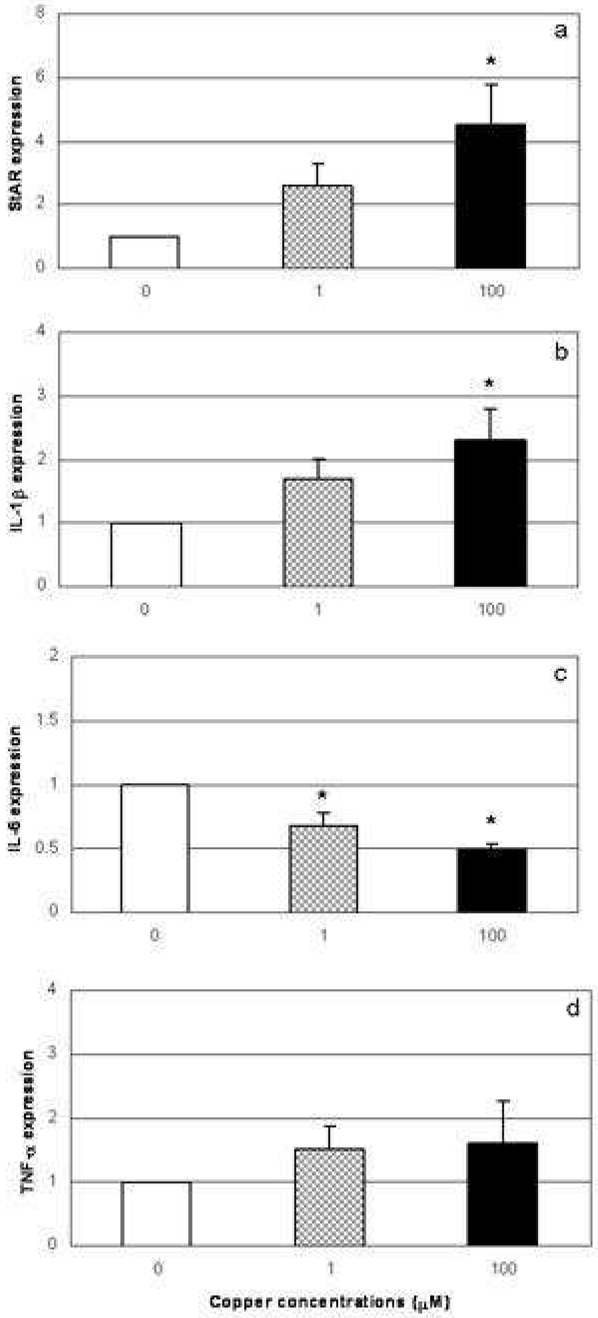
Total RNA was isolated from head kidney using Tri Reagent and reverse transcribed with Superscript III™ reverse transcriptase (Invitrogen SA, Spain). The cDNA from head kidney preparations were used for qPCR analysis. Total volume (20 μL) of every reaction contained 500 nM of each amplification primer, 10 μL of 2X SYBR Green PCR Mix and 5 μL of a 1:25 dilution of cDNA (1:500 for 18S determination). Products were amplified in an iCycler iQ™ Real Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA). Controls lacking cDNA were included. The real-time analysis consisted of 1 cycle of 95 ºC for 5 min, 40 cycles of 95 ºC for 10 s and 60 ºC for 30 s, 1 cycle of 95 ºC for 1 min, 1 cycle of 55 ºC for 1 min, and a melting curve of 81 cycles (from 55 ºC to 95 ºC). All samples were run in triplicate. CT (threshold cycle) values for each sample were expressed as "fold differences", calculated relative to untreated controls and normalized to endogenous control 18S rRNA.

Cell Viability: Cell viability was determined using the Trypan blue exclusion method. The number of viable and dead cells was counted. The Cu concentrations tested were 0, 1µM, 10µM, 100µM, 1mM and 10mM. Incubation times were: 1, 2, 3 and 4 h. The choice of metal concentration range and exposure time was made based on previous in vitro studies concerning fish head kidney cells treated with Cu (Gagnon et al., 2006).

Statistical Analysis: Statistica software (StatSoft, Inc., Tulsa, OK) was used for statistical analyses. All the data were first tested for normality and homogeneity of variance to meet statistical demands. ANOVA analysis was used to compare results between groups, followed by LSD test. Differences from control: $^*P<0.05$.

Results: The head kidney cells incubated with the concentrations ranging from 1 µM up to 1 mM of Cu during 1, 2, 3 or 4 h had viability above 90%. Though, the highest tested concentration induced almost 100% mortality. Considering these results, the Cu concentrations adopted for the present study (1 and 100 µM) are in a sublethal range for seabream head kidney cells.

The levels of StAR and IL-1β gene expression were increased in seabream head kidney cells after 1 h incubation with 1 or 100 µM of Cu. However, only the highest concentration (100µM) showed statistical significance when compared to control (Fig. 1a and 1b). The dose-response relation was obvious for StAR and IL-1 β, being more evident for StAR, since its increment at 100 µM Cu were around 5 times the control, whereas at 1 µM Cu the increase was around 2 times; despite this difference, there was no significant difference between treatments. Conversely, IL-6 mRNA levels (Fig. 1c) were significantly decreased in head kidney cells incubated with 1 or 100 µM Cu. The results also revealed that TNF-α expression (Fig. 1d) was not different from control levels in both treated groups, although showing a slight increase.



[Copper effects]

Fig. 1: In vitro effects of 1h Cu incubation (1 and 100 µM) on StAR (a), IL-1β (b), IL-6 (c) and TNF-α (d) mRNA expression in head kidney cells of seabream. mRNA expression was analyzed by quantitative real-time PCR and normalized to 18S rRNA. Values represent the means and SE (n=4). Differences from control: * $P<0.05$.

Conclusions:

- Cell viability was above 90% in a concentration range up to 1 mM; however, 10 mM induced almost 100% mortality, revealing that head kidney cells of seabream are highly susceptible to this concentration of Cu. These values are different when compared with other species, suggesting that cell resistance to Cu exposure may depend on the fish species (Gagnon et al., 2006).
- StAR mRNA expression was induced in head kidney cells incubated with Cu, revealing the sensitivity

of this tissue to chemical agents, as it is for biological agents. Thus, previous findings showed a clear StAR increase in seabream head kidney cells incubated 1 h with ACTH (Castillo et al., 2008). The present StAR increase may be also due to a stimulation of the corticosteroid cells by the presence of Cu, since it was previously observed that fish exposed to Cu displayed high cortisol levels in plasma (Teles et al., 2005).

- Concerning cytokine mRNA expression, IL-1β was increased, that may be related with its action as an "early cytokine" in the inflammatory response. IL-6 is a secondary cytokine in the inflammatory response and the present results show a decrease in expression, especially for the highest Cu concentration.
- Cytokines can elicit regulatory effects on corticosteroid secretion, and thus the association between IL-1β and the increase in StAR mRNA expression could be hypothesized.
- Results suggest that Cu affected endocrine as well as immune parameters in head kidney cells of seabream, though more work is needed to establish clear interactions between StAR and cytokines expression in order to clarify Cu effects at these levels.

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P 4

Comparison of two bioassays for testing sediment samples of sites contaminated by mining activities

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Introduction: Determination of the contaminant content is not enough to evaluate the toxic effects or to characterise contaminated sites, because it does not reflect the ecotoxicological danger in the environment and does not provide information on the effects of the chemical compounds. To estimate the risk of contaminants, chemical methods need to be complemented with biological methods. Ecotoxicological testing may be a useful approach for assessing the toxicity as a complement to chemical analysis. The aim of this study was to evaluate the sensitivity and applicability of two bioassays representing multiple trophic levels, for the preliminary ecotoxicological screening of sediments from sites contaminated by mining activities: a chronic toxicity test with the ostracod *Heterocypris incongruens* and a phytotoxicity test using *Lepidium sativum, Sorghum saccharatum* and *Sinapis alba* seeds.

Material and methods: For this study, 27 sediment samples were collected from the Sierra Minera (Murcia, SE Spain) and were air-dried and sieved to <2 mm for general analytical determinations using the following methods: pH using 1:5 suspensions in water and KCl; EC(dS m^{-1}) and Eh (mV) in the filtered 1:5 suspension. Equivalent calcium carbonate and organic matter content were also determined in soil samples. To determine the total metal content (Pb, Zn, Cd and As), samples were digested in an acid solution in a Milestone ETHOS PLUSmicrowave. The Zn content was determined by flame atomic absorption spectrometry. The Pb and Cd content was determined by electrothermal atomization atomic absorption spectrometry (ETAAS) and As content was determined by atomic fluorescence spectrometry. Standard reference materials (SRM 2709 San Joaquin Soil and SRM 2711Montana Soil) were used to check the reliability of the method.

The Phytotoxkit® test measures the decrease in (or the absence of) seed germination and of the growth of the young roots after 3 days of exposure of seeds of selected higher plants to a contaminated matrix compared with the controls germinated in a reference soil. The plants selected for the Phytotoxkit® microbiotest were: the monocotyl *Sorghum saccharatum* (Sorgho) and the dicotyls *Lepidium sativum* (Garden cress) and *Sinapis alba* (mustard) (Phytotoxkit®, 2004). The percent inhibition of seed germination (SG) and root growth inhibition (RI) for each plant were calculated.

Ostracodtoxkit® test consists of placing freshly hatched ostracod neonates in multiwell cups in 2 ml synthetic freshwater, with 300 µl sediment and $3x10^7$ algal cells (*Selenastrum capricornutum*). After 6 days, incubation at 25 ºC in darkness, the mortality of test organisms was determined (Ostracodtoxkit® FTM, 2001) and growth inhibition was calculated.

Results and discussion: Soil samples showed a mean pH value of 6.0 in water and 5.7 in KCl. The EC varied from 1.0 dS m^{-1} to 56.2 dS m^{-1}, with a mean value of 17.7 dS m^{-1}. The mean value for Pb was 0.84 mg kg^{-1}, 10593 mg $kg^{-1}$for Zn, 23.18 mg kg^{-1} for Cd and 0.16 mg kg^{-1} for As.

As regards the phytotoxicity test, an influence on seed germination was observed (Figure 1). In S9, S10, S18, S21, S22, S25 and S26 no seeds germinated for any plant species. In samples S5, S20, and S23 the seed germination inhibition percentage ranged from 60% to 90% and samples S1, S3, S4, S12, S14, S16, S17, S19, S24, S27 and S30 showed percentages of seed germination inhibition lower than 20%.When root length was evaluated, a significant inhibition of root growth was noticed. In the case of S9, S10, S18, S21, S22, S23, S25 and S26, inhibition ranged from 80% to 100%. In the case of S5, S11, S12, S13, S16, S20 and S30 it ranged from 30% to 60% while the other soil samples showed a root length inhibition percentage lower than 30%. Samples S17 and S27 were an exception and had a stimulating effect on root growth.

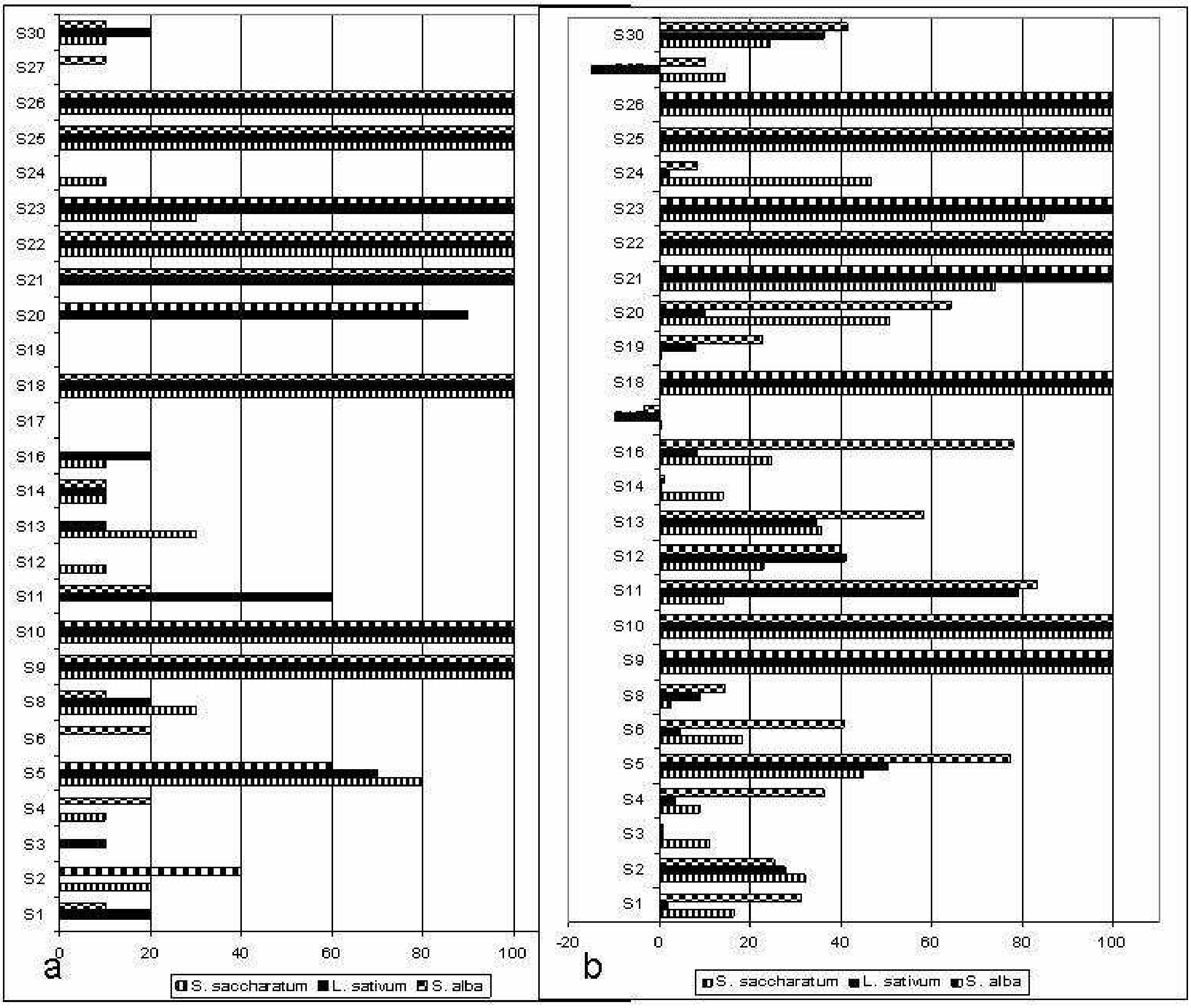
Correlation analysis between heavy metal concentration, soil characteristics and plant test results showed that growth inhibition in the plant species was negatively correlated with pH and calcium carbonate content and was positively correlated with Eh, EC and total arsenic content in soil samples.

The mortality data for *H. incongruens* for the 26 sediment samples is plotted in Figure 2. From this figure it is clear that the majority of the samples induced 100% mortality in the ostracods. For the three soil samples (S6, S12 and S17) the mortality scores ranged from more than 50% to 70%. Only five sediment samples (S4, S14, S16, S19 and S27) killed less than 20% of ostracods during the 6-day exposure period. The percent of growth inhibition for the ostracod in the studied samples, with mortality lower than 100%, showed that significant growth inhibition was noted. In S3, S14, S19 and S27, mortality was lower than 20%. Samples S4 and S16 showed the highest mortality percentage (28%-50%). Correlation analysis showed that the mortality of ostracods was positively correlated with Eh and EC and negatively correlated with pH and calcium carbonate content.

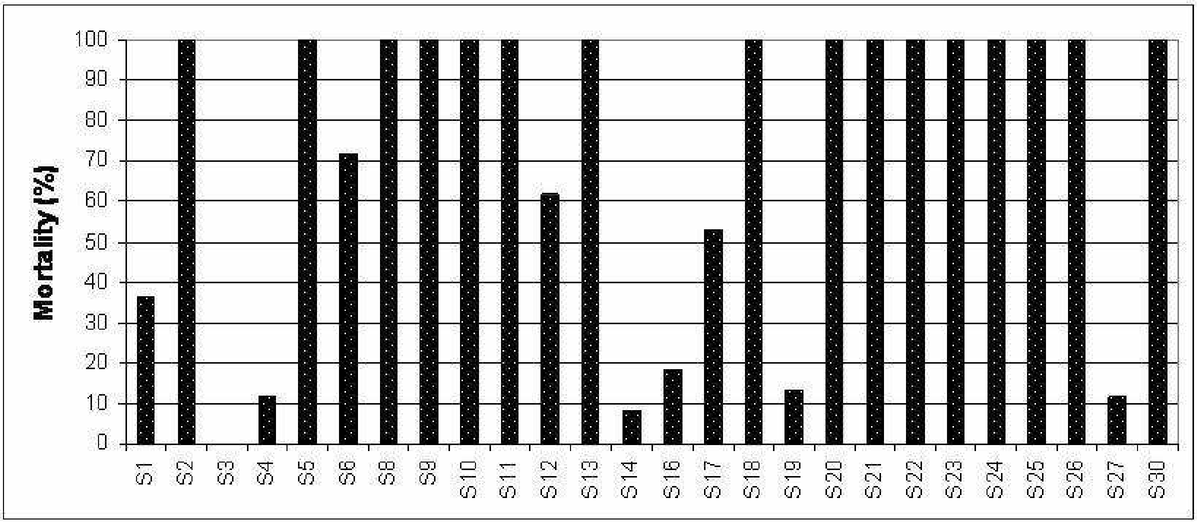
Conclusion: Of the bioassays used in this study, the ostracod test was the most sensitive. The assays carried out showed greater sensitivity to Cd followed by As and, lastly Pb and Zn.

References: Ostracodtoxkit FTM (2001) Chronic direct contact toxicity test for freshwater sediments. Standard Operational Procedure, Creasel, Deinze, Belgium

Phytotoxkit (2004) Seed germination and early growth microbiotest with higher plants. Standard Operation Procedure. Nazareth, Belgium



[Graph1]
Figure 1: Mortality (a) and growth inhibition (b) in plant species



[Graph2]
Figure 2: Mortality % in H. incongruens

P 5

A multibiomarker approach to assess environmental pollution in the Lagoon of Venice (Italy). II: the crab Carcinus aestuarii as sentinel species

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The multibiomarker approach is nowadays deemed the most suitable strategy in biomonitoring studies, better enabling the assessment of the effects of mixtures of contaminants in aquatic environments. In the present study three biomarkers were evaluated in male crabs Carcinus aestuarii collected in November 2007 in 4 sites of the Lagoon of Venice: Valle di Brenta and Cà Roman, located in the southern lagoon basin, Marghera and Palude del Monte, in the northern one. Acetylcholinesterase (AChE) activity (in gills and haemolymph), as a biomarker of exposure to neurotoxic compounds, vitellogenin (Vg)-like protein levels (in digestive gland, haemolymph and gonads), as a biomarker of exposure to estrogenic compounds, and ethoxyresorufin O-deethylase (EROD) activity (in digestive gland), as a biomarker of exoposure to several planar compounds such as polycyclic aromatic hydrocarbons (PAHs) and dioxin-like polychlorinated biphenyls (PCBs), were measured. The results showed significantly lower AChE activity in the haemolymph of crabs from Marghera, near the industrial area of Porto Marghera, and from Palude del Monte, used as a licensed area for clam culture. This suggests an exposure of animals to neurotoxic compounds originating from industrial effluents and from the urban area of the proximate city of Venice. Moreover, it has to be noted that Palude del Monte is located not far from a riverine outlet, possibly conveying agricultural wastewaters. Conversely, significantly higher AChE activity values were observed at Valle di Brenta, in the southern Lagoon. The same pattern of AChE activity was observed in crab gills, although significant differences among sites were not detected. Significantly higher levels of Vg-like proteins were recorded in the digestive gland and haemolymph of crabs collected at Palude del Monte. The lowest values were observed in digestive glands of crabs from Cà Roman, next to the lagoon inlet, and in haemolymph of crabs from Valle di Brenta, close to mainland. Higher EROD activity, although not significantly different, was observed in digestive glands of crabs from Cà Roman, where intense fishing and ship traffic occur. Since for all biomarkers no significant difference between Valle di Brenta and Cà Roman was found, data from both sites were pooled together and compared with those, similarly pooled, from the northern sites of Marghera and Palude del Monte. Interestingly, this comparison showed a significantly better condition for the southern basin if referring to Vg-like protein level and AChE activity, while a better condition was observed for the northern basin in relation to EROD activity. In the interpretation of this last result, the extremely high variability of EROD activity in crabs from the northern sites, compared to the more homogeneous situation for the southern sites, needs to be taken into account. Certainly, further investigation is required to check for this variability. In conclusion, considering all the results obtained, similar pollution levels in the monitored sites are suggested, despite the differences observed between northern and southern basin in relation to each specific biomarker, thus reflecting different conditions of anthropogenic impact. When comparing the biomarker responses in crabs and clams (see Matozzo et al., this Congress), indications can be gathered for using crabs, mobile and mainly carnivorous organisms, as sentinels for wider geographical areas in biomonitoring studies.

P 6

Arsenic solubility and plant uptake from soils affected by mining activities in Murcia, Spain

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Introduction: In spite of being one of the most pollutant toxic which can be found, As is widely distributed in nature (Kabata-Pendias, 2007). Arsenic is naturally found in plants, but its concentration in vegetal tissue seldom exceeds 1 mg kg^{-1} (Adriano, 2001). The tolerance of this element in plants is normally 2 mg kg^{-1} (Kabata-Pendias, 2007) and it can be absorbed from soils or from the matter deposited in their leaves. The most serious risk posed by As is associated with the forms that are biologically available for absorption, or "bioavailable" to plants. The aim of this study was to assess arsenic concentrations and solubility in soils, and its uptake by plant species growing in an area strongly affected by mining activities in Murcia (SE, Spain). For this, the effect of different chemical reagents which can be used to determine the bioavailability of this element in different environmental conditions, i.e: natural or potential conditions and especially those which are bioavailable by the plant were studied.

Material and methods: For this study, fourteen samples were collected in the surrounding area of Sierra Minera and Portman Bay (Murcia, SE Spain). Samples of five plant species (*Limonium carthaginens, Arthrocnemum macrostachyum, Dittrichia viscosa, Glaucium flavum and Zygophyllum fabago*), which grow naturally in the studied area, were collected. Samples were air dried and sieved to < 2mm for general analytical determinations. The pH was determine in a 1:5 suspension of soil pure water and in a 1M KCl solution (Peech, 1965) using a Crison GLP21 pH meter. Electrical conductivity (EC) (dS/m at 25ºC) was measured using a Crison GlP31 meter in the extracts obtained by filtering the 1:5 suspensions through a 0.45 µm cellulose acetate disk filter. Equivalent calcium carbonate (%) was determined by the volumetric method using a Bernard calcimeter previously calibrated against Na_2CO_3 (Hulseman, 1996; Muller and Gatsner, 1971). The organic matter content was also measured (Duchaufour, 1970).The mineralogical composition was studied by X-Ray diffraction (XRD), using a Philips PW3040 diffractometer using the Cu-Kα. Line. To determine the As content, soil samples were first ground to a fine powder using an agate ball mill. Samples were placed in Teflon vessels and 5 ml of concentrated HF acid solution, 200µl of concentrated HNO_3 acid solution and 5ml of water were added. Fresh vegetable samples, were separated into root and aboveground biomass and then lyophilized. 200mg of lyophilized vegetal tissue were placed in Teflon vessels with 3 ml of water, 2ml of concentrated H_20_2 and 5ml of concentrated HNO_3 acid solution. When digestion was complete (15 minutes at 1000W in a Milestone ETHOS PLUS microwave), the samples were transferred to a volumetric flask and brought to 50ml.

The bioavailable concentrations of As in plant samples were calculated by different soil chemical extraction methods; deionized water, 0.1M HNO_3 (Walter W.Wenzel et al.,2001), 0.5N $HNaCO_3$ (Olsen, S.R. et al., 1954), oxidizable-organic matter (3rd step BCR), 0.5 HCl (an adaptation of the chemical extraction method of Adriano 2001), 1N ammonium acetate (Adriano 2001), 0.05M $(NNH_4)_2 SO_4$ (Walter W.Wenzel et al.,2001), 0.005M DTPA (Lindsay and Norvell,1978) and Mehra-Jackson extraction.

Arsenic levels were obtained by using atomic fluorescence spectrometry with an automated continuous flow hydride generation (HG-AFS) spectrometer (PSA Millenium Excalibur 10055). The reliability of the results was verified by analysing two standard reference materials (SRM 2711 Montana Soil and SRM 1515 Apple leaves).

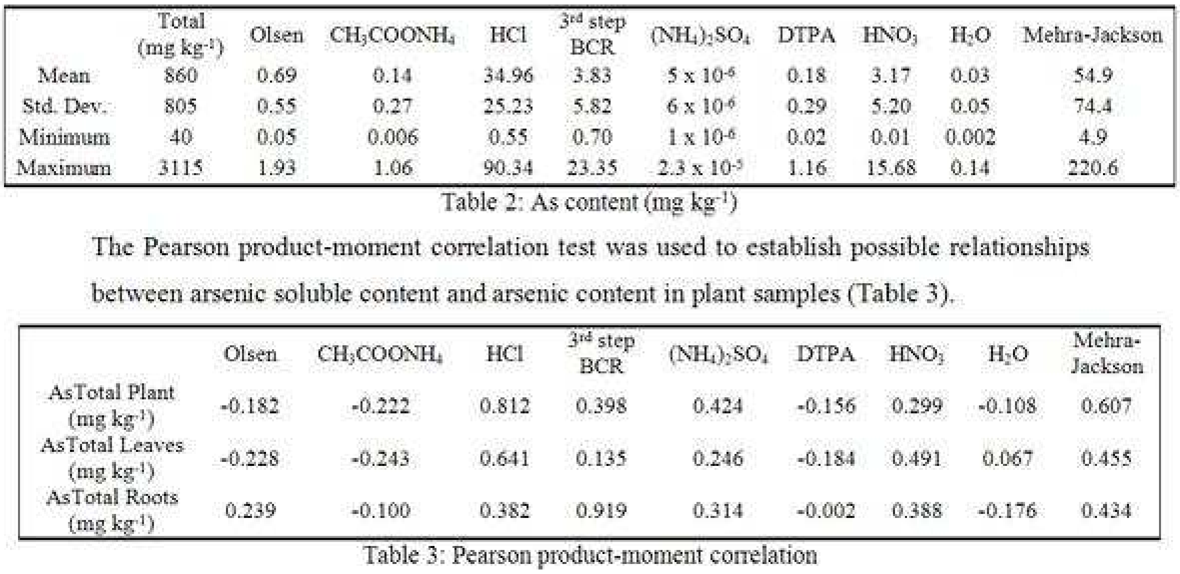
Results and discussion: The general characteristics of soil samples are shown in Table 1. Samples showed pH average values closely to neutrality. Most samples showed a very low organic matter percentage. Electrical conductivity was considerably low in most samples as well calcium carbonate content. The mineralogical analysis showed that the main minerals were quartz, muscovite, kaolinite and illite, while the minority minerals were products of mining activities (iron oxides and hydroxides, siderite, jarosite and gypsum), calcite and feldspars.

	pH H_20	pH KCl	EC (mS/cm)	$CaCO_3$	O.M.
Mean	7.08	6.79	1.80	4.18	0.84
Std. Dev.	1.36	1.37	1.97	8.24	0.45
Minimum	4.25	4.15	0.20	0.00	0.20
Maximum	9.06	8.52	7.40	28.70	1.90

Table 1: Soils characteristics

[1]

The concentration of soluble arsenic was determined after 9 different extractions and mean value for arsenic in these extractions was 0.69 mg/kg for Olsen extraction, 0.14 mg/kg for extractable fraction by ammonium acetate, 35 mg/kg extracted by HCl acid, 3.8 mg/kg in the oxidisable-organic matter and sulphides fraction, 0.2 mg/kg for DTPA extraction, 3.2 mg/kg extracted by HNO_3 and 55 mg/kg for extractable fraction by Mehra-Jackson. Finally, water and ammonium sulphate extraction showed 0.03 and 5×10^{-6} mg/kg respectively. Details of these measusements are given in Table 2. Plant samples were collected in all the soils studied. The samples represented 5 species: *Limonium carthaginens, Arthrocnemum macrostachyum, Dittrichia viscosa, Glaucium flavum and Zygophyllum fabago.* Average content in roots and leaves was 18.1 mg/kg and 23.5 mg/kg respectively.



	Total (mg kg^{-1})	Olsen	CH_3COONH_4	HCl	3rd step BCR	$(NH_4)_2SO_4$	DTPA	HNO_3	H_2O	Mehra-Jackson
Mean	860	0.69	0.14	34.96	3.83	5×10^{-6}	0.18	3.17	0.03	54.9
Std. Dev.	805	0.55	0.27	25.23	5.82	6×10^{-6}	0.29	5.20	0.05	74.4
Minimum	40	0.05	0.006	0.55	0.70	1×10^{-6}	0.02	0.01	0.002	4.9
Maximum	3115	1.93	1.06	90.34	23.35	2.3×10^{-5}	1.16	15.68	0.14	220.6

Table 2: As content (mg kg^{-1})

The Pearson product-moment correlation test was used to establish possible relationships between arsenic soluble content and arsenic content in plant samples (Table 3).

	Olsen	CH_3COONH_4	HCl	3rd step BCR	$(NH_4)_2SO_4$	DTPA	HNO_3	H_2O	Mehra-Jackson
AsTotal Plant (mg kg^{-1})	-0.182	-0.222	0.812	0.398	0.424	-0.156	0.299	-0.108	0.607
AsTotal Leaves (mg kg^{-1})	-0.228	-0.243	0.641	0.135	0.246	-0.184	0.491	0.067	0.455
AsTotal Roots (mg kg^{-1})	0.239	-0.100	0.382	0.919	0.314	-0.002	0.388	-0.176	0.434

Table 3: Pearson product-moment correlation

[2]

Conclusion: The results showed that the arsenic content in roots was positively correlated with the oxidizable-organic matter and sulfides fraction (third step sequential extraction procedure). Arsenic concentration in leaves was positively correlated with the arsenic extracted by HCl and HNO_3, with the oxidizable-organic matter and sulfides fraction and with the arsenic extracted by Mehra-Jackson extraction. According to our results, As is accumulated in the leaves of the plants and is linked with iron oxides of these soils affected by mining activities.

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Bioaccessibility of metals in waste mining materials from Portman Bay, SE Spain

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Introduction

A study on metal (Zn, Pb, Cd, Cu and As) mobilization and analysis of the health risk represented by ingestion from contaminated sediments in Portman Bay (SE Spain) was carried out. This zone has suffered a great impact from mining activity, since million tons of mine tailings were dumped into the bay for a long period, giving as a result the filling of the bay with them.

The long-term deposition of metals in soils and sediments can lead to their accumulation and transport, while their toxicity depends on the mobility and bioavailability of a significant fraction of the metals (Adriano, 2001). The ingestion of contaminated soil particles by grazing animals or young children may well represent a special exposure pathway for Pb, Cd and other hazardous metals (Schroder et al., 2003). Contaminant bioavailability, rather than total content, is increasingly used as a key indicator of the potential risk that contaminants pose to both environmental and human health. The National Research Council (NRC, 2003) defines bioavailability in terms of the fraction of the total contaminant mass in soil and sediment that is readily available to receptor organisms, including human and ecological organisms. The oral bioaccessibility is the fraction that is soluble in the gastrointestinal environment and is available for absorption (Ruby et al., 1999; Mercier et al., 2002). The aim of this study was to determine the bioaccessibility of Zn, Pb, Cd, Cu and As ,and the extent to which bioaccessibility is influenced by mineralogy in materials from this old mining site as an indicator of the potential risk that metals pose to both environmental and human health.

Material and methods

General analytical determinations (pH, particle size, organic matter, equivalent calcium carbonate content and mineralogical composition) were carried out to characterize the samples.
For bioaccessibility tests 20 samples were collected, air dried and sieved to < 250 µm size fraction since this size it is believed to represent the fraction of soil that is most likely to adhere to human hands and become ingested during hand-to-mouth activity. The pH was determined in a 1:5 suspension of soil and equivalent calcium carbonate (%) was determined by the volumetric method using a Bernard calcimeter. The organic matter content was also measured (Duchaufour, 1970). The mineralogical composition was studied by X-ray diffraction (XRD), using a Philips PW3040 diffractometer with Cu-Kα.
To determine the total metal content, the samples were digested in a Milestone ETHOS PLUS microwave, using HF and concentrated HNO_3. Zn, Pb, Cu and Cd contents were determined by electrothermal atomization atomic absorption spectrometry using an Unicam 929 AASpectometer, while As was analysed by HG- AFS using an automated continuous flow hydride generation spectrometer (PSA Millenium Excalibur 10055). Standard reference materials (SRM 2709 San Joaquin Soil and SRM 2711 Montana Soil) were used to check the extraction efficiency of the method.
To assess bioaccessibility, the gastric solution was prepared according to the Standard Operating Procedure (SOP) developed by the Solubility/Bioavailability Research Consortium (SBRC).

Results

A summary of the physico-chemical characteristics of the sediments studied is given in Table 1. On the other hand, Table 2 summarizes the results obtained in the determination of the total metal contents as well as the corresponding bioavailable fractions.
The mineralogical composition (Figure 1) corresponds to materials which have suffered a supergenic oxidation process which has been influenced by the presence of sea water. Unaltered minerals (philosilicates, quartz, sulphides and magnetite) as well as those resulting of oxidation and carbonatation processes (iron oxihydroxides, hematite, siderite, jarosite and hidrated sulphates as copiapite, bianchite, coquimbite and gypsum).
The results showed that the fraction of metals dissolved by the *in vitro* procedure is less than 100% in the gastric solution. The solubility of each metal under synthetic fluids depends on its chemical speciation and binding capacity to different soil and sediment materials. Mineral associations, different alteration states and sorption/desorption processes play an important role in the gastrointestinal bioavailavility of metals.
The data here obtained can be incorporated to the general protocole of risk analysis by ingestion applied to contaminated sites. This could be of interest since when risk assessments are adjusted to account for lower site-specific bioavailability, the resulting increase in cleanup levels can substantially reduce the cost of remediation in some cases.

	pH	EC dSm^{-1}	% OM	%$CaCO_3$	< 250 µm	< 63µm	< 5µm
Max.	8.2	2.80	54.6	15.4	100	52.0	35.0
Min.	0.70	0.10	4.5	n.d	11.4	2.2	0.4
Mean	4.9	0.30	23.2	0.5	81.9	25.4	12.4

Table 1. Physico-chemical characteristics of sediments samples

	As		Pb		Cu		Zn		Cd	
	Total	Bioac.	Total	Bioac.	Total	Bioac.	Total	Bioac.	Total	Bioac.
Max.	6600	59.0	8000	1500	300	26.2	27900	4100	35	15.0
Min.	284	0.8	1400	1010	20	0.8	1200	1030	10	6.4
Mean	580	4.0	3200	1060	52	8.0	8900	1800	18	5.1

Table 2. Total and bioaccessible heavy metal content (mg.Kg^{-1})

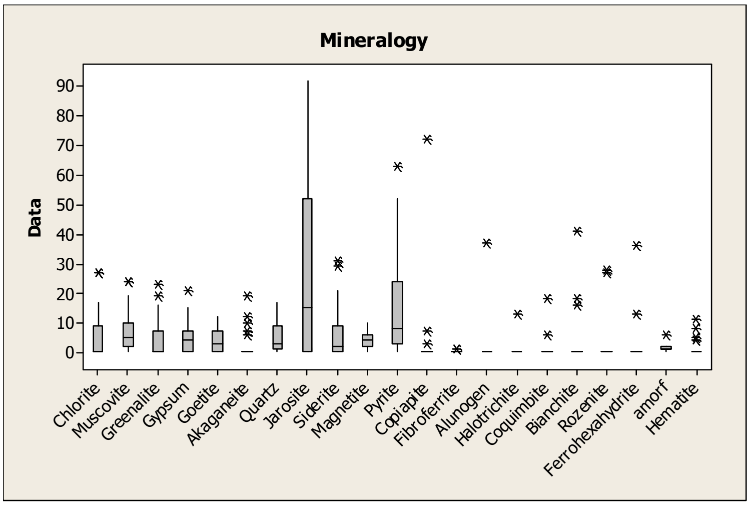

Figure1. Box plot showing the mineralogy of the samples.

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Needle volatile as a key factor in the plant-insect interaction for some Albanian pine species

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Volatile compounds emitted by plants have been shown to act as a key factor in recognition and selection of hosts by many pest insects (Devotka et al., 1990) and needles are the principal mediators of biotic interactions with folivorous insects, such as Thaumatopoea pityocampa Denn & Schiff (Hódar et al., 2002).

This study focused on evaluating the chemical composition of essential oils obtained from six Pinus species (Pinus nigra, P. marittima, P. halepenis, P. radiata, P. pinea, P. sylvestris), growing in natural forests in Northern Albania.

More than 90 components were detected, characterized and quantified as oil constituents on the basis of their retention times and their mass spectra. The six pine species represented widely different monoterpene compositions. The different level of infestation shown in these trees is highly correlated with their chemical profiles. The quantification of the essential oils was obtained using a Hewlett Packard (HP 6890) gas chromatograph equipped with DB-5 (60mx 0.25mm) fused silica column. Monoterpenes and sesquiterpenes were dominant in the majority of the tested species. Within the monoterpene domain, α-pinene, β-pinene, δ-3 carene, limonene, myrcene, terpinolene and β-phellandrene were the most abundant monoterpenes detected (Tsitsimpikou et al., 2001; Petrakis et al., 2005), whereas the sesquitepene composition was mostly dominated by germacrene -d, δ-cadinene, β - caryophyllene and α- humulene. Some of these compounds are well known as attractants and some as repellents for the insect Thaumatopoea pityocampa Denn & Schiff (Loreto et al., 2000; Hódar et al., 2002; Petrakis et al., 2005). Since volatile monoterpenes are a key factor in host-plant interactions, these results should lead to further investigations that would help in unfolding the ecological role of a complex mixture of secondary metabolites.

Key words: Pinus, α-pinene, β -pinene, β-caryophyllene, Germacrene-D.

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Tsitsimpikou C, Pertakis P, Ortiz P, Harvala C, Roussis V (2001). Volatile Needle Terpenoids of Six Pinus Species. J. Essent. Oil Res. 13: 174-178

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Niddle volatile as a key factor in the plant-insect interaction, for some Albanan pine species

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Key words: Pinus, α -pinene, β -pinene, β-caryophyllene, Germacrene-D.

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The inhibition effect of heavy metals on COD removal in UASB reactor

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Upflow anaerobic sludge blankent reactor (UASB) within the anaerobic processes is widely used in order to remove chemical oxygen demand (COD) and product biogas. Although heavy metal inhibition to the conventional anaerobic treatment processes has been reported, limited studies have been carried out for UASB reactor.

In this study, inhibition effects of copper (Cu) and zinc (Zn) metal ions on COD removal efficiency were investigated in UASB reactor and heavy metal inhibition concentrations for hydraulic retention time (HRT) of 12 and 24 hour were determined. Influent COD concentration to the reactor was kept constant as 3000 mg/l by using glucose as primary carbon source. Heavy metal concentrations given into reactor for both of them were 1,5,10,15,20 mg/l.

The influent Zn and Cu concentrations at which the COD removal efficiency decreased to 50%(CI,50) for HRT 12 and 24 hours were found to be 13.37-13.71 mgZn/l and 12.67-13.01 mgCu/l, respectively.

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Study of using fungi's rot to biodegrade soils contaminated with pesticides

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In several of biological technology solutions used to degrade soil contaminated with pesticides, the solution of using the white rot fungus is becoming the best choice of researchers due to friendly-environment feature itself . However, the method of using white rot fungus in order to degrade several substances of pesticide in soil such as dichlodipheyltrichloetan (DDT), 2,4- dichlophenoxyacetic acid (2,4- D) and 2,4,5-trichlophenoxyacetic acid (2,4,5-T) has just been experimented in laboratory. Several rotten fungi are mixed into contaminated solid in terms of liquid. There are still few research projects which mentioned the use of fungi´s rot (one of agricultural wastes) for clearing pesticide-contaminated soil.

In this report, we would like to introduce the initial research step of using the rots of some kinds of fungi containing enzyme which belongs to lignin-degrading system, for example, *Pleurotus Sajor Caju* (Japan), *Pleurotus Ostreatus* (Vietnam), *Hipsizygus Marmoreus* (Japan), *Lentined Edodes* (Vietnam) in order to degrade contaminated soil of 2,4-D, 2,4,5-T and DDT.

To assess the usage of fungi's rot to treat pesticide-contaminated soil we need to research on the characteristics of enzyme system to biodegrade lignin in fungi´s rot, which is shown in Figure 1 and Table 1.

Table 2, Table 3, Table 4 are the experiment results to assess the effectiveness of using fungi´s rot for degrading soil contaminated with 2,4-D, 2,4,5-T and DDT. The above results shows that the productivity of degrading organic substances depends on several factors including the ratio of rots and soil, the activity of enzyme degrading lignin.

The result shows that, in order to biodegrade substances of pesticide containing chloride such as 2,4-D and 2,4,5-T, it is need to use several fungi namely Pleurotus Ostreatus, Pleurotus Sajor Caju, Hipsizygus Marmoreus and Lentinud Edodes by the method of mixing directly their rots and contaminated soil. With the proportion of 1/5 fungi´s rot contaminated soils (by mass) can degrade 85% - 95% or 50% - 70% initial DDT after 30 days of treatments and enzyme activity degradation lignin has special effect on performance of degrading contaminated soil. Because the change of activity of enzyme degrading lignin (LiP, MnP) during the time of fungus' cultivation, the choice of suitable condition and time for the purpose of having best samples of rots with high enzyme activity will have an important effect to contaminated soil's degradation.

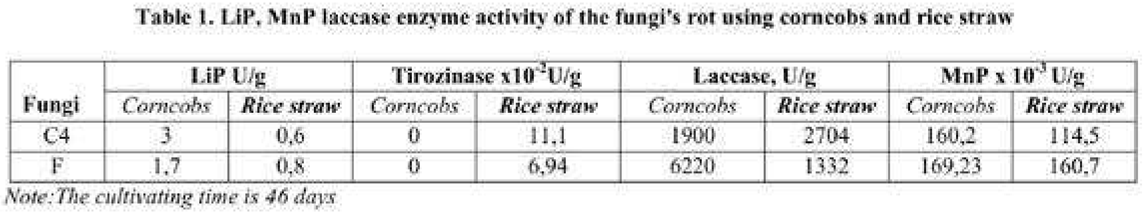

Table 1. LiP, MnP laccase enzyme activity of the fungi's rot using corncobs and rice straw

Fungi	LiP U/g		Tirozinase x10^{-2}U/g		Laccase, U/g		MnP x 10^{-3} U/g	
	Corncobs	***Rice straw***	*Corncobs*	***Rice straw***	*Corncobs*	***Rice straw***	*Corncobs*	***Rice straw***
C4	3	0,6	0	11,1	1900	2704	160,2	114,5
F	1,7	0,8	0	6,94	6220	1332	169,23	160,7

Note:The cultivating time is 46 days

[1]

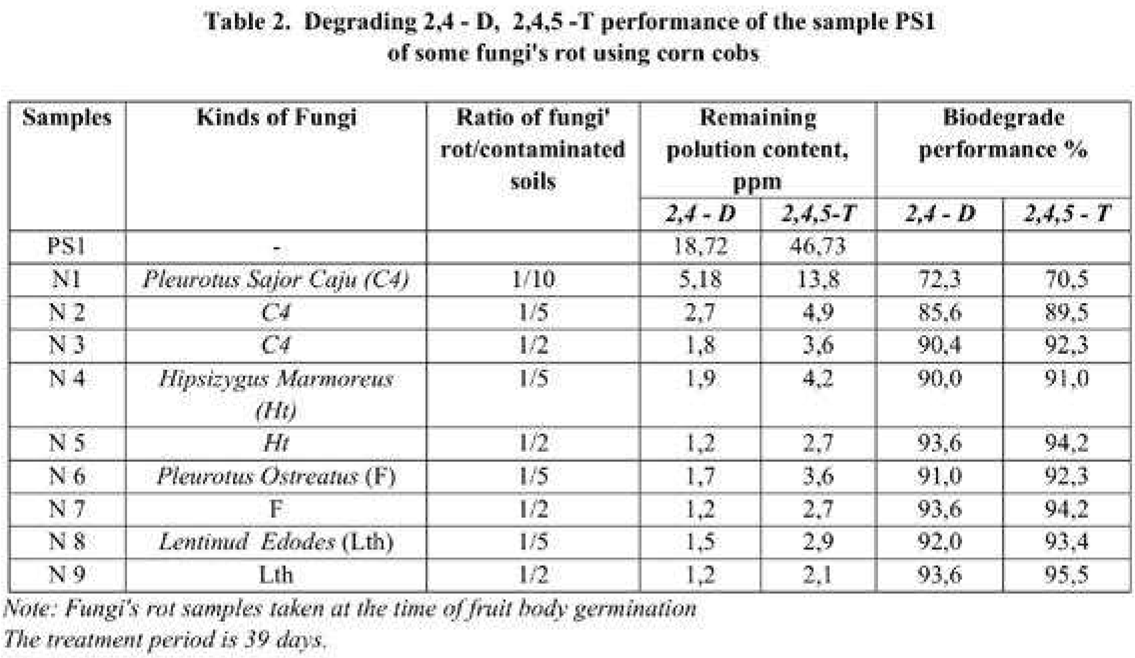

Table 2. Degrading 2,4 - D, 2,4,5 -T performance of the sample PS1 of some fungi's rot using corn cobs

Samples	Kinds of Fungi	Ratio of fungi' rot/contaminated soils	Remaining polution content, ppm		Biodegrade performance %	
			2,4 - D	*2,4,5-T*	*2,4 - D*	*2,4,5 - T*
PS1	-		18,72	46,73		
N1	*Pleurotus Sajor Caju (C4)*	1/10	5,18	13,8	72,3	70,5
N 2	*C4*	1/5	2,7	4,9	85,6	89,5
N 3	*C4*	1/2	1,8	3,6	90,4	92,3
N 4	*Hipsizygus Marmoreus (Ht)*	1/5	1,9	4,2	90,0	91,0
N 5	*Ht*	1/2	1,2	2,7	93,6	94,2
N 6	*Pleurotus Ostreatus* (F)	1/5	1,7	3,6	91,0	92,3
N 7	F	1/2	1,2	2,7	93,6	94,2
N 8	*Lentinud Edodes* (Lth)	1/5	1,5	2,9	92,0	93,4
N 9	Lth	1/2	1,2	2,1	93,6	95,5

Note: Fungi's rot samples taken at the time of fruit body germination
The treatment period is 39 days.

[2]

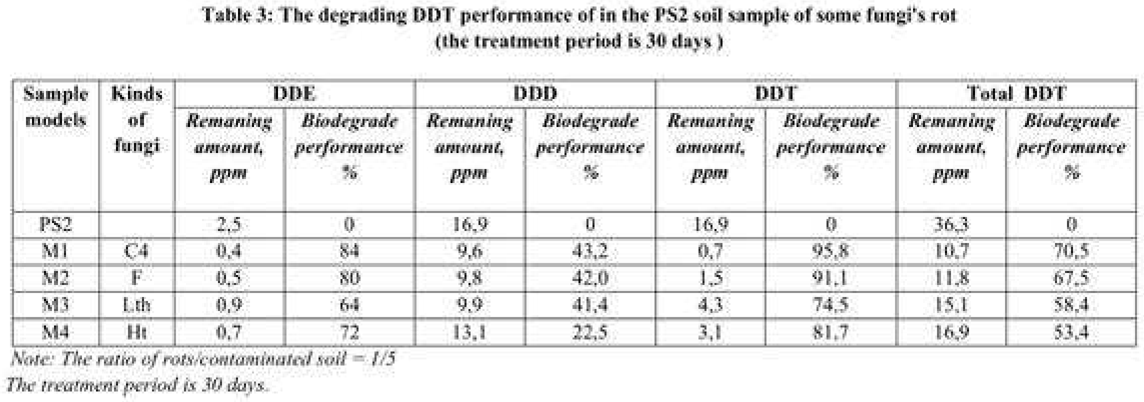

Table 3: The degrading DDT performance of in the PS2 soil sample of some fungi's rot (the treatment period is 30 days)

Sample models	Kinds of fungi	DDE		DDD		DDT		Total DDT	
		Remaning amount, ppm	*Biodegrade performance %*	*Remaning amount, ppm*	*Biodegrade performance %*	*Remaning amount, ppm*	*Biodegrade performance %*	*Remaning amount, ppm*	*Biodegrade performance %*
PS2		2,5	0	16,9	0	16,9	0	36,3	0
M1	C4	0,4	84	9,6	43,2	0,7	95,8	10,7	70,5
M2	F	0,5	80	9,8	42,0	1,5	91,1	11,8	67,5
M3	Lth	0,9	64	9,9	41,4	4,3	74,5	15,1	58,4
M4	Ht	0,7	72	13,1	22,5	3,1	81,7	16,9	53,4

Note: The ratio of rots/contaminated soil = 1/5
The treatment period is 30 days.

[3]

Table 4: The relation between biodegration performance of 2,4-D, 2,4,5-T and lignin degradation enzyme activity in fungi's rots

No	Kinds of fungi	Remaining amount of contaminated soil, ppm		Biodegrade performance %		Enzyme activity, U/g		
		2,4 - D	*2,4,5 – T*	*2,4 - D*	*2,4,5 – T*	*LiP*	*Laccase*	*MnPx10^{-3}*
1	PS3	20,8	36,4	0	0	-	-	-
2	F	3,81	8,32	81,70	77,1	4,6	18100	111,13
3	C4	1,68	3,66	91.9	89,9	11,2	11420	2292
4	C4+F	1,98	5,64	90,5	84,5	5,9	18460	158,45
5	Ht	4,37	8,5	79,0	76,6	2,5	34	47,84
6	Lth	7,5	16,7	63,9	54,1	2,2	0	10,6

Note: The ratio of rost/contaminated soil = 1/2
The treatment period is 30 days.

[4]

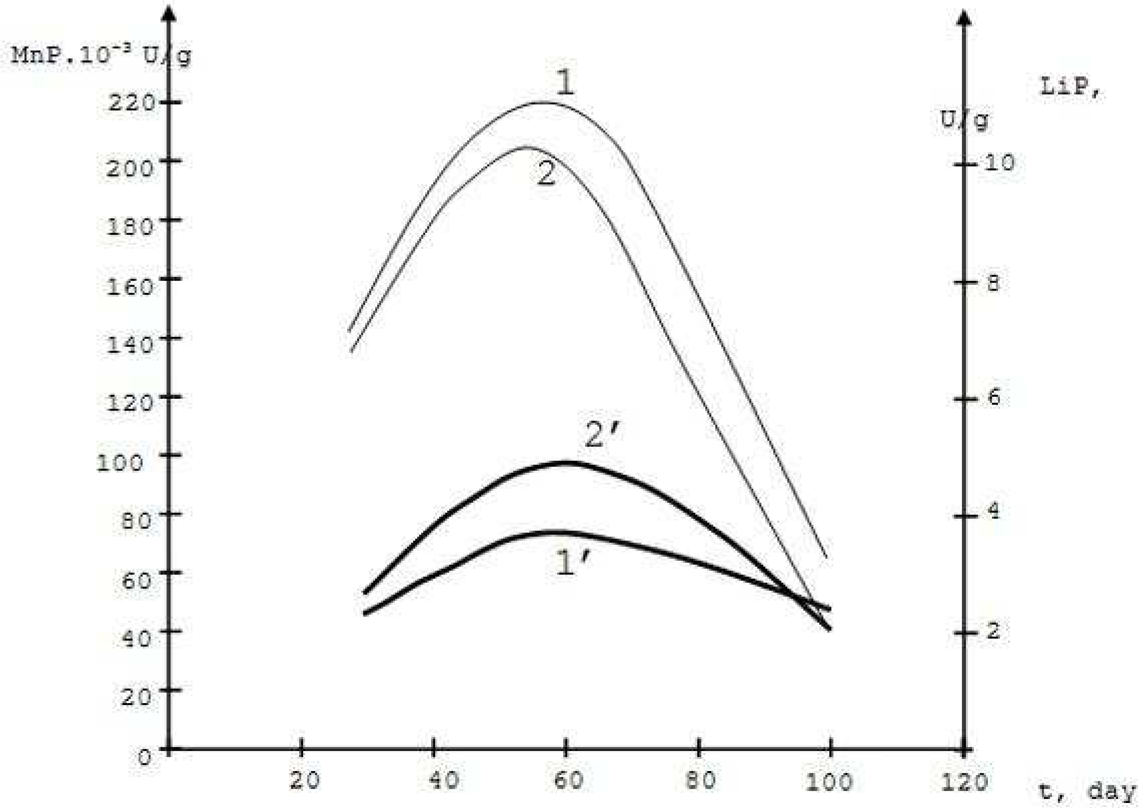

Figure 1: The changes of enzyme activity MnP (1,2), Lip (1', 2') during the period of fungi cultivation
1, 1' – Fungus's rot C4
2, 2' – Fungus's rot F

[5]

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Uranium (VI) sorption onto goethite (α-FeOOH): Synthesis and characterization

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Iron oxy-hydroxides such as ferrihydrite, magnetite, hematite, and goethite show high specific surface area and their surface are positively charged at low and circum neutral pH values. Goethite is a common and thermodynamically stable iron oxide in most soils. Under natural pH values it is highly insoluble and has a net positive surface charge making it an effective sorbent of metal anion complexes from aqueous solutions. α-FeOOH is also very important from the academic standpoint. In colloid and surface chemistry, the synthetic α-FeOOH particles are often used as a model adsorbent in fundamental studies of the adsorption/desorption phenomena. These minerals play a main role in the retardation of anionic contaminants, such as uranium, arsenic, molybdenum, and antimony. Goethite was chosen for this study as it is amongst the most common of iron oxyhydroxides, and is found to have a strong affinity for adsorption of the uranium (VI). Goethite has a well characterized crystal structure, and was easily prepared under laboratory conditions
In this work, our objective was the study of the sorption of uranium(VI) ions on goethite from aqueous solutions. The acicular α-FeOOH particles were synthesized from Fe(NO3)3 solutions at a very high pH using potassium hydroxide as a precipitating agent. The relative importance of test parameters like solution pH, contact time, temperature and concentration of adsorbate on adsorption performance of goethite for U(VI) ion were studied. Typical adsorption isotherms (Langmuir, Freundlich, Dubinin-Raduskevich) were determined for the mechanism of sorption process. Also the thermodynamic constants (ΔH°, $\Delta S^\circ$and ΔG°) were calculated. Goethite was characterized by X-ray powder diffraction, scanning electron microscope (SEM) and BET specific surface area (SSA).

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Ecogenic nephrometalotoxicoses

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Mechanisms of the pathology of kidneys development, induced by heavy metals (HÌ), represent indefatigable interest. It is determined by the role of HÌ as global environmental pollutants and complex, diverse character of their interaction with nephrones. Ecologically dependent defeats of kidneys are characterized by a number of features:
1. Plural sources of the toxicant receipts (air, alimentary, water,);
2. influence of small dozes and concentration of HÌ;
3. long exposition - development of chronic defeats after the long latent period (powerful reserves, long adaptation and indemnification);
4. primary defeat of proximal tubes with the subsequent involving in pathological process glomerular component;
5. presence of functional changes on the part of other organs and the systems quite often masking the nephropathy. Methodical approaches for the early revealing of metallonephrotoxicoses with the use of sensitive integrated and selective chemical and biological markers are developed. Necessity of biochemical and physiological monitoring among contingents of the population living in areas of increased risk of occurrence ecogenic diseases on the basis of use of HÌ contents of maintenance in biosubstrats and carrying out of functional loading tests is proved. The technique is approved on contingents of workers of transport and communication, the population of seaside cities with high risk of the metallonephropathies development, caused occupationally and ecologically caused contact with ÒÌ.

P 14

Fire retardants in ecotoxicology of polymers

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Small-scale researches of 378 synthetic and natural (celulosocotaining) polymeric materials on combustion products toxicity are carried out. They included quantitative parameters definition of chemical substances migrating, and their toxicity for white mice and rats. It is shown, that fire retardants bring the essential contribution to display of dangerous properties by the investigated compositions. At processed by fire retardants polymers the combustion products toxicity in 48 % of cases raised, in 29 % - was reduced and in 22 % of cases remained at an initial level. At control group of materials toxic properties are caused mainly by carbon oxide (II) in more than 50 % of cases. Toxicity exposed with fire retardants compositions is directly connected to used additives: halogenated hydrocarbons, polybrominated diphenyl ethers, di-and monoammony phosphates. This was coordinated to the blood carboxyhaemoglobin level in the exposed animals, and also character of biochemical changes in cells and tissues. Made researches have shown, that the data on toxicity of the chemical substances migrating from polymers accepted in traditional toxicology, are not enough for the degree of combustion products toxicity and danger estimation. They should be added with results of tests recommended by ISO, IEÑ or regulated by national standards.

P 15

Effect traits of endocrine disruptors in aquatic non-vertebrate ecosystems

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Whereas potential impact on vertebrates has been intensively investigated on single species base, the number of studies dealing with hazard of ED to non-vertebrate organisms or higher tier test systems is limited. For an increasing number of invertebrates hormone systems have been shown, but in most cases precise knowledge about these is still outstanding. Steroid hormone systems have been published to occur in several invertebrate taxa (nematodes, molluscs, annelides, crustaceans, insects, echinoderms, tunicates). Basic features of these systems are phylogenetically conserved in invertebrates and vertebrates, whereas the details concerning the hormone regulated pathways diverge. Not surprisingly, these hormone systems are susceptible to endocrine disruptors, although again knowledge is still poor.

Although providing indispensable basic information, data from tests with single species are of limited significance. For protection of an ecosystem, its components have to be considered comprehensively, even though some of them might be affected only indirectly. However, ED effects have hardly been investigated in model ecosystems. A major reason may be the general difficulty to assign effects in ecotoxicological studies to endocrine disrupting activities, as the chemicals may have other (non-endocrine) toxic properties as well. This problem is enhanced by the complexity of model ecosystems and by the deficiency of knowledge about hormone systems of invertebrates. However, non-vertebrate organisms, e.g. phyto- and zooplankton, but also benthic organisms as nematodes, represent key groups in aquatic ecosystems. As a result, it is often arguable whether ecosystem alteration due to a specific substance is really caused by ED activity, or whether ED activity in general is really a major threat for the environment.

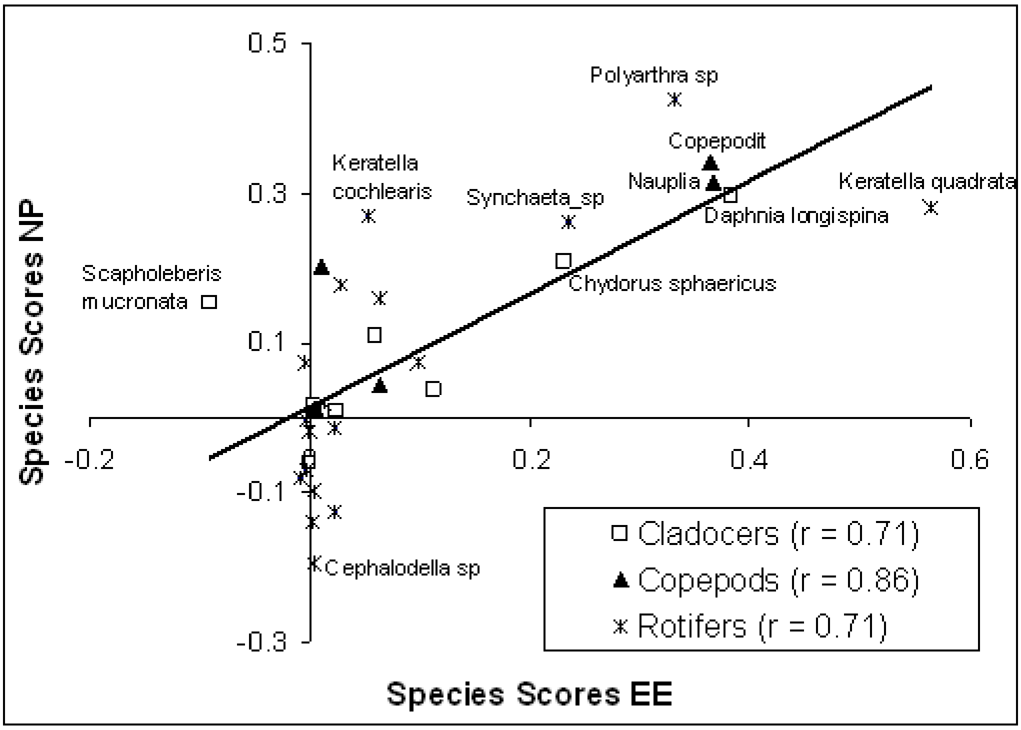
We thus conducted still water microcosm studies containing no vertebrates with two estrogenic EDs. One (nonylphenol, NP) is known to be endocrine disruptive, but has also relevant non-endocrine toxicity. The other (17α-ethinylestradiol, EE) has high estrogenic activity but almost no clear non-endocrine toxicity (for an overview see Schramm et al., 2008), making it a suitable reference chemical for estrogenic endocrine disruptors. We thus regard it as a positive reference.

The effects of NP and EE in these studies have been published in detail (Hense et al., 2003, 2008, Severin et al., 2003, Schramm et al., 2008). Both chemicals affected all investigated organism groups, including (indirectly) phytoplankton. Here, we focus on the following questions:

Are there indications for effect traits which could be specific for endocrine disruption? Might it thus be possible to estimate, whether found effects of other substances are probably due to endocrine disruptive or other toxic properties?

Material and methods: Microcosms constructed of cylindrical, bottom-closed stainless steel containers (∅ 80 cm, height 60 cm) were filled with sediment (10 cm) and water (230 L) from an oligo-mesotrophic littoral area of Lake Ammersee, Germany. NP and EE (both Sigma-Aldrich, Seelze, Germany) were added using controlled-release devices made from semipermeable low-density polyethylene (LDPE) tubes of different lengths. 7 microcosms were continuously exposed to NP (6 to EE) for 5-6 weeks, 9 (6) served as controls. Zoo- and phytoplankton samples were taken before, during and some weeks after end of treatment, fixed with sugar-formalin solution (resp. Lugol for phytoplankton), identified and counted in a stereo- (respectively inverse-) microscope. Species number and composition (principal response curve, PRC), abundances, diversity (Shannon/Weaver index), and biomass were calculated. For more details see elsewhere (Hense et al., 2003, 2008, Severin et al., 2003, Schramm et al., 2008).

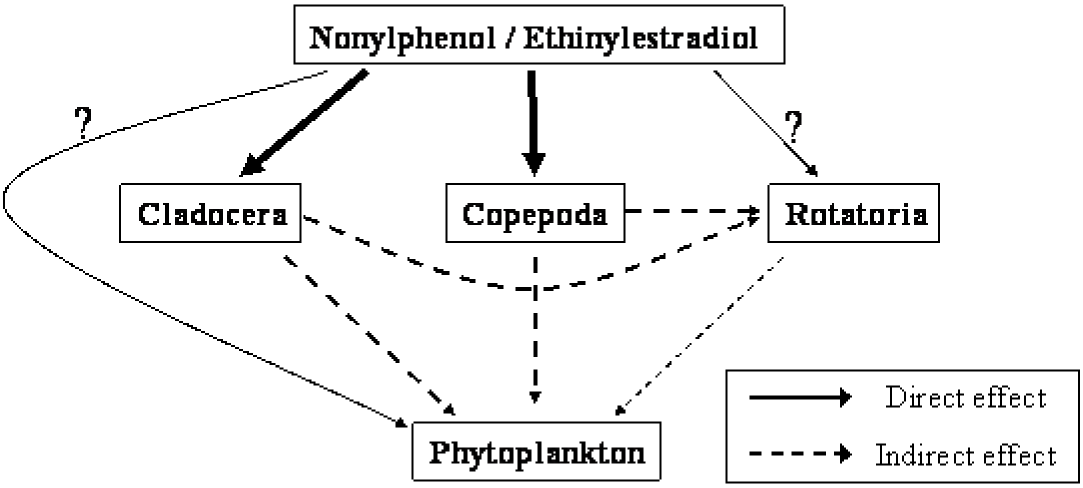
Results and discussion: Maximum concentrations reached were between 9 and 120 µg/L for NP, resp. between 49 and 724 ng/L for EE. After the removal of the controlled-release devices, concentrations declined rapidly, reaching limits of determination within a few days. Most of the global end points (species number, diversity, biomass) were affected for zoo- and phytoplankton, however common reaction patterns of both test chemicals were difficult to derive. Regarding zooplankton, cladocers and copepods (especially juveniles) showed tendency to decrease after exposure to both substances, whereas rotifers reacted ambiguously. In contrast, phytoplankton seemed to be promoted (biomass, number of taxa). In a next step, we analyzing effects of EE and NP on species level by species scores (PRC). For zooplankton species occurring in both test periods a certain degree of consistence regarding the most affected taxa was found (Fig. 1). For phytoplankton this consistency seemed to be lower.



[fig.1]

Fig. 1: Comparison of species scores, calculated on species level for zooplankton by PRC.

Based on measured data and multivariate statistics, as well as ecological and toxicological arguments, our results indicate a similar mode of effect for both substances on the plankton community (Fig. 2). Cladocers and copepods (juveniles) were affected directly. For rotifers, direct as well as indirect (weaker suppression by crustaceans?) influences may have occurred. Although different species of phytoplankton showed varying reactions, generally the phytoplankton community seemed to have benefited from the shifts in zooplankton, probably due to a decreased feeding pressure.



[fig.2]
Fig. 2: Probable main effect net of EE and NP on plankton community

Although a final statement is not possible based on the preliminary results, there are hints that endocrine disruptors, especially those interfering with steroid hormone systems, may affect plankton communities in a specific way. Groups as crustacean, which are known to have (steroid) hormone systems and are reported in single species test to be sensitive for endocrine disruptors (for literature Schramm et al., 2008, Severin et al., 2003), decline. In contrast phytoplankton increases, usually with a certain time lag. However, this hypothesis has be confirmed in further tests and molecular investigations. It has to be kept in mind that several other, as far as known non-endocrine chemicals exhibit a similar ecotoxicological reaction pattern (for literature see Schramm et al., 2008).
In our studies, the effect strength of the NP was lower than that of EE, although the used NP concentration was about three orders of magnitude higher. However, - similarily to EE - first effects occurred at concentration below most EC values reported in single species toxicity test. Furthermore, (juvenile) copepoda and cladocera belonged to the most affected groups, phytoplankton was later (indirectly) affected, and species scores of PRC calculated on species level showed similarities to EE. All in all, these results at least fit to a (partly) endocrine disruptive causation.

Literature: Hense, B.A., Jüttner, I., Welzl, G., Severin, G.F., Pfister, G., Behechti, A., Schramm, K.-W., 2003. Environ. Toxicol. Chem. 22, 2727-2732.
Hense BA, Jaser W, Welzl G, Pfister G, Wöhler-Moorhoff GF, Schramm K-W., 2008. Ecotox. Environ. Safe. 69, 453-465
Schramm K-W, Jaser W, Welzl G, Pfister G, Wöhler-Moorhoff GF, Hense BA., 2008. Ecotox. Environ. Safe. 69, 437-452.
Severin, G.F., Welzl, G., Jüttner, I., Pfister, G., Schramm, K.-W., 2003. Environ. Toxicol. Chem. 22, 2733-2738.

P 16

The use of selected genotoxicity assays in D. labrax and L. aurata: A validation study in Aveiro Coastal Lagoon (Portugal)

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Aims: Previous chemical analyses displayed the presence of polycyclic aromatic hydrocarbons (PAHs), organometallic compounds and heavy metal, as an indication of water quality deterioration in some critical areas of Aveiro estuarine system. Therefore, the current study was designed to assess the genotoxic effects, as 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels and erythrocytic nuclear abnormalities (ENA) frequency in Dicentrarchus labrax and Liza aurata caught, at different Aveiro lagoon sites (Torreira-TOR, Barra-BAR, Gafanha-GAF, Rio Novo do Príncipe-RIO, Laranjo-LAR and Vagos-VAG) in Autumn 2005. In addition, it was also intended to evaluate the fish suitability for environmental health assessment in a multi-pollution context.

Methods:

1. Sampling: Juvenile sea bass (Dicentrarchus labrax) and golden grey mullet (Liza aurata) were caught in the Autumn of 2005 using fishing role and a traditional beach-seine net named "chincha", respectively. Juvenile specimens were selected on the basis of their size. Biometrical parameters, such as fish weight and total length ranged from 18.71 - 41.96 g and 12.9 - 16.5 cm, respectively for L. aurata as well as 18.71 - 41.96 g and 12.9 - 16.5 cm for D. labrax. Blood was collected from the posterior cardinal vein using a heparinized Pasteur pipette and used for smear preparation and plasma separation using an Eppendorf centrifuge (14 000 rpm). The sampling sites in Aveiro Lagoon were selected on a geographic distribution basis along the main channels and at the lagoon entrance, taking into account the various type and sources of contamination as well as the selection of a (theoretically) unpolluted reference point. Thus, the sampling sites were: TOR as a reference point at the stretch of the longest channel (S. Jacinto-Ovar) having non-point pollution inputs and far from the main polluting sources; BAR at the initial part of the Mira channel, close to the sea-side lagoon entrance and subjected to a considerable naval traffic; GAF situated in the vicinity of a deep-sea fishing port and dry-docks, also connected with the main channel coming from Aveiro city and receiving domestic discharges; RIO located at the terminal area of the Vouga river, 6.5 km distant from a pulp/paper mill effluent outlet, was exposed to its discharges since nearly five decades up to 2000; LAR presenting a well described mercury contamination gradient (mainly $HgCl_2$) as a result of chlor-alkali plant discharges; VAG at the terminal part of the Ílhavo channel, receiving municipal and domestic effluents.
2. 8-OHdG assay: 8-OHdG was quantified in blood plasma through a competitive enzyme-linked immunosorbent assay using an IBL (Hamburg) kit.
3. ENA assay: Blood smears were fixed with methanol during 10 minutes and stained with Giemsa (5%) during 30 minutes. The nuclear abnormalities were scored in 1000 mature erythrocytes sample per fish, according to the criteria of Schmid (1976), Carrasco et al. (1990) and Smith (1990), adapted by Pacheco and Santos (1996). According to these authors, nuclear lesions were scored into one of the following categories: micronuclei, lobed nuclei, dumbbell shaped or segmented nuclei and kidney shaped nuclei. The final result was expressed as the mean value (%) of the sum for all the individual lesions observed.
4. Statistical analysis: Results are expressed as means ± SE (standard error) corresponding to experimental groups of six fish (n=6). The experimental data were tested first for normality and homogeneity of variance to meet statistical demands. ANOVA analysis was used to compare the results of the various fish groups, followed by the Tukey´s test in order to compare results between fish groups (Zar, 1999) and the significance of the results was ascertained at $p < 0.05$.

Results: Higher 8-OHdG plasma level was found in D. labrax caught at GAF as well as in L. aurata caught at LAR which demonstrates the presence of oxidative agents in environmental water of those sites. In addiction, the biological differences among these two fish species, such as mobility, feeding behaviour and susceptibility to DNA damage (e.g. defence mechanisms and DNA repair) may explain the observed differences. Moreover, the contamination pattern, i.e. the possibility of the presence of different pollutants in GAF and LAR, as causative agents for these observed genotoxic effects should also be considered. Concerning ENA frequency, elevated values were only found in L. aurata caught at VAG. No significant sea bass ENA increase was observed in the present autumn survey despite the presence of a variety of contaminants, such as PAHs, TBTs, resin acids and heavy metals in Aveiro Lagoon system. According to Pacheco and Santos (2002) ENA assay may be inadequate for situations of intense contamination since some disturbances of genotoxicity expression may occur. These disturbances were confirmed to depend on the balance between an increased splenic erythrocytic catabolism and reduced erythropoiesis rate (Pacheco and Santos, 2005).

Conclusions: The current genotoxic results demonstrate the risk to fish populations exists at GAF, LAR and VAG sites reflecting different contamination patterns as a result of a mixture of pollutants from point and non point sources. However, the circumstantial absence of significant sea bass ENA increase should be interpreted with some precaution. The observed L. aurata oxidative DNA damage (8-OHdG) is not correlated with ENA occurrence. D. labrax and L. aurata revealed to be particularly appropriate species for genotoxicity monitoring of heterogeneous genotoxic contamination in coastal ecosystems.

P 17

Endocrine and metabolic seasonal responses of D. labrax L. captured from a contaminated estuary (Aveiro Lagoon, Portugal)

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Aims: Bearing in mind the suitability of juvenile sea bass (Dicentrarchus labrax) as a bioindicator species, in the present study, a battery of 6 biomarkers was evaluated on D. labrax caught seasonally (spring, summer, autumn and winter 2006) at six different polluted Aveiro Lagoon locations. The endocrine function was assessed by measuring plasma levels of cortisol, thyroid-stimulating hormone (TSH), free triiodothyronine (T3), free thyroxine (T4). The intermediary metabolism (processes involved on the energy extraction from nutrient molecules) was also evaluated as plasma glucose and lactate levels. The integrated biomarker response (IBR) index was used to improve the discriminatory power of the adopted multi-biomarker strategy.

Methods:

1- Sampling: Juvenile sea bass (D. labrax) were caught in the spring, summer, autumn and winter 2006. Juvenile specimens were selected on the basis of their size. The sampling sites in Aveiro Lagoon were selected on a geographic distribution basis along the main channels and at the lagoon entrance, taking into account the various type and sources of contamination as well as the selection of a (theoretically) unpolluted reference point. Thus, considered sites were: Torreira (TOR) as a reference point at the stretch of the longest channel (S. Jacinto-Ovar) having non-point pollution inputs and far from the main polluting sources; Barra (BAR) at the initial part of the Mira channel, close to the sea-side lagoon entrance and subjected to a considerable naval traffic; Gafanha (GAF) situated in the vicinity of a deep-sea fishing port and dry-docks, also connected with the main channel coming from Aveiro city and receiving domestic discharges; Rio Novo do Principe (RIO) located at the terminal area of the Vouga river, 6.5 km distant from a pulp/paper mill effluent outlet, was exposed to its discharges since nearly five decades up to 2000; Laranjo (LAR) presenting a well described mercury contamination gradient (mainly $HgCl_2$) as a result of chlor-alkali plant discharges; Vagos (VAG) at the terminal part of the Ílhavo channel, receiving municipal and domestic effluents.

2- Hormonal responses measurement: The determinations of cortisol, TSH, T3 and T4 were performed in plasma, using diagnostic ELISA direct immunoenzymatic kits (Diametra, Italy). The absorbance was measured at 450 nm in a microplate reader (ASYS Hitech). The cortisol in the sample competes with horseradish peroxidase (HRP)-cortisol for binding onto the limited number of anti-cortisol sites in the microplate wells. The enzyme substrate (H_2O_2) and the TMB (3,3´, 5,5´ tetramethylbenzidine)-substrate are added, and after an appropriate time has elapsed for maximum color development, the enzyme reaction is stopped and the absorbances are determined. Cortisol concentration in the sample is calculated based on a series of standards and the color intensity is inversely proportional to the cortisol concentration in the sample. The methods for free T3 and free T4 follow the same principles of the cortisol test, requiring immobilized T3 or T4 antibodies, as well as HRP-T3 or HRP-T4 conjugates. Concerning TSH, an antibody specific to the h-chain of TSH molecule is immobilized on microwell plates and other antibodies to the TSH molecule are conjugated with HRP. TSH from the sample is bound to the plates. The enzymatic reaction is proportional to the amount of TSH in the sample.

3- Intermediary metabolism responses measurement: Plasma glucose was measured spectrophotometrically (340 nm) according to the method modified from Banauch et al. (1975), based on the quantification of NADH after a glucose oxidation catalysed by the glucose-dehydrogenase. The quantity of NADH formed is proportional to the glucose concentration. Plasma lactate levels were determined spectrophotometrically (340 nm) according to the method modified from Noll (1974) using LDH, ALT and NAD, measuring the NADH appearance.

4- Integrated biomarker response (IBR): A method for combining all the measured biomarker responses into one general ‘‘stress index’’ termed ‘‘Integrated Biomarker Response’’ (IBR) (Beliaeff and Burgeot, 2002; Broeg and Lehtonen, 2006) was applied.

5- Statistical analysis: Results are expressed as means ± SE (standard error) corresponding to experimental groups of six fish (n=6). The experimental data were tested first for normality and homogeneity of variance to meet statistical demands. Multi-factorial ANOVA analysis was used to compare the results of the various fish groups, organs and seasons followed by the Tukey´s test in order to compare results between fish groups (Zar, 1999) and the significance of the results was

ascertained at $p < 0.05$.
Results: Regarding to hormonal responses, decreased T3 and cortisol was found in fish collected from LAR in spring. Taking into consideration that successful cortisol release relies on a functionally intact HPI axis, our data indicate a HPI axis impairment. High T4 levels were also found at BAR in the previous season. In a first analysis, the absence of alterations on plasma TSH levels observed in our study could suggest that pituitary was not affected by the environmental mixtures present in all sites studied. As our knowledge, xenobiotic-induced alterations in plasma thyroid hormones may be a result of a variety of mechanisms, corresponding to changes on thyroid status and/or alterations upstream or downstream of the thyroid hormones release. These mechanisms include alterations on the hypothalamus and/or pituitary status (Alkindi et al., 1996); biosynthesis and secretion steps of T3 and T4 (Capen, 1997), uptake by peripheral tissues, hepatic 5-monodeiodinase activity (Waring et al., 1996), or hormone catabolism and clearance rates (Saito et al., 1991; Hontela et al., 1995).
Intermediary metabolism responses, namely plasma glucose were higher in D. labrax caught at BAR in spring and lower in winter. Lactate was also found elevated at LAR and VAG in spring, however in autumn, the plasma lactate levels decreased at RIO and LAR, when compared to TOR. The plasma glucose increase observed in our study can be regarded as an expectable result since it may be used by fish as an energy substrate to carry out adjustment processes to environmental stressors. On the other hand, plasma lactate decrease may be explained by its stimulated use in liver gluconeogenesis. Thus, plasma lactate decrease as well as the glucose increase may be attributed to an increased liver cortisol uptake and a consequent gluconeogenic inducing action. Hence, the previously mentioned parameters may also be useful tools in monitoring the impact of anthropogenic stressors on fish. Considering the combination of T3, cortisol and lactate levels, high IBR was found to GAF in spring and summer, as well as RIO and LAR in autumn and winter, respectively.
Conclusions: (1) The adopted stress parameters allied to a seasonally survey strategy are recommended for other future environmental monitoring assessments. D. labrax responses to mixtures of xenobiotics are complex and the type of interaction in a particular mixture can vary with the evaluated biological response.
(2) The observed D. labrax alterations in glucose and lactate levels as well as in plasma concentrations of the hormones reflect endocrine alterations; therefore, fish physiological competence to cope with environmental stressors can be affected. These investigation findings provide a rational use of adopted multi-biomarkers applied to D. labrax and can be successfully employed for biomonitoring anthropogenic contamination in both Atlantic and Mediterranean European coastal waters.

P 18

Molecularly imprinted polymers as a tool for detection of benzimidazol derivatives

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Benomyl is a benzimidazol fungicide used in control of various crop pathogens. Residue analysis is mainly based on the detection of its main metabolite carbendazim. The method relies on liquid liquid extraction involving different steps of transfer from aqueous to organic solution. This procedure generates losses and reduced rates of recovery in addition to the noise caused by the coextractive components from the matrix.
Alternative methods are described in the literature such as solid phase extraction (SPE) and solid phase micro-extraction (SPME). These methods offer rapid extraction based on the adsorption from the solution (SPE) or from the solution and gaseous phase (SPME) of pesticides or pollutants in general. However, they lack of selectivity and coextractive compounds limit their performance.
Molecularly imprinted polymers (MIPs) offer better alternative by adsorbing in preference the target molecules. This is obtained by the recognition of the molecule by a polymer prepared in presence of template which can be the molecule of interest or belonging to the same chemical family.
In this work we have tested a MIP and compared results with SPE technique. The adsorbent was synthesized using metacrilic acid as monomer, toluene as porogen, EGDMA(ethylen glicol

dimetacrilate) as cross linker, AIBN (azobis isobutironitrile) as initiator and benomyl as template. After grinding the solid obtained, the granules were sieved to 3 fractions (∅ <45 µm, 45< ∅<100 µm and ∅ >100 µm) for SPE tests. MIPs were extracted by Soxhlet to ensure the best elimination of template. Retention of benomyl was tested with MIP compared with NIP (not imprinted polymer). Imprinted Factor (IF), that represents the ratio KMIP/KNIP, was constant whatever the % of organic eluent (methanol) was used. Test of specific recognition showed good performance with benomyl compared with other pesticides tested, tiophanate methyl and dicofol.

Test with SPE were performed using 300 mg C18 cartridge, conditioned with 10 mL of methanol followed with 10 mL of distilled water. Cartridges of MIP were filled with 300 mg of adsorbent and conditioned in the same manner that of SPE. Solutions tested were enriched separately with benomyl, carbendazim and thiophanate methyl. The mean recoveries of benomyl obtained were estimated to ca. 62 and 56.4 % for SPE and MIPs respectively. Results with MIPs were improved to 74.5 when the cartriges were eluetd with a 1mL chlorofom followed by 4 mL methanol.

The low value obtained for benomyl could be due to use of carbendazim as reference. This could have introduced a mis-evaluation of residue issued from the degradation of benomyl. More investigations are needed to confirm this results and assess the effect of other parameters such as solvent, size of particules , the amount of solvent ...)

P 19

Halotolerant Penicillium as a tool for saline wastewater remediation processes

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Many industries are known to generate wastewater enriched in phenolic compounds. These include petrochemicals, basic organic chemical manufacture, coal refining, pharmaceutical and tanning. Consequently, these compounds are commonly encountered in industrial effluents and surface water. Due to its high toxicity as shown by ecotoxicological studies, several methods have been reported for the removal of these pollutants from wastewater. Additionally to this toxicity problem some of these industrial effluents are likely to generate highly saline wastewaters. The discharge of such wastewaters containing at the same time phenol and phenolic compounds and high salinity without prior treatment is known to negatively affect the aquatic life, agriculture and potable water. Biological treatment with halotolerant/halophilic microorganisms is considered advantageous over the other physical and chemical methods as it leads to complete mineralization of phenolic compounds, is one of the safest, least costly and most socially acceptable. Halotolerant microorganisms are well known for their great versatility to remove pollutants, under saline and non saline conditions. Penicillium chrysogenum is an economically important ascomycete used as producer of penicillin. However, little attention has been paid to the ability of this microorganism to transform or metabolize compounds that are pollutants. This article presents a different approach to a classic problem. It employs an individual test on marine organism of trophic level 2 to validate bioremediation process by halotolerant fungus. A biodegradation process is an effective method of remediation if the toxicity of the system decreases.

The purpose of this study was to compare biodegradation of single phenol and hydroquinone at high initial substrate levels by halotolerant strain, P. chrysogenum CLONA2, under saline, adapted with either phenol or hydroquinone, in a batch system, and to study the inhibitory and enhanced effect during the biodegradation of phenolic compounds. Single and binary substrate experiments were performed.

P. chrysogenum CLONA2 was able to utilize hydroquinone as the sole carbon source over a wide range of hydroquinone concentrations at 5.8 % (w/v) NaCl. There were differences in detoxification efficiencies of phenolic compounds. In the case of batch with phenol or mixture (hydroquinone plus phenol) hydroquinone was most rapidly detoxified. The acute toxicity of phenol and hydroquinone, individually and in combination, to larvae of the Artemia franciscana has been verified after and before bioremediation process with P. chrysogenum CLONA2. The two phenolic compounds tested in this study have different LC50, and consequently exhibit different toxicity. Hydroquinone was more toxic than phenol. After remediation process no toxicity was detected in samples from batch with phenol, hydroquinone or mixture. Attend to all findings present in this study the possibility to use this halotolerant strain of P. chrysogenum for detoxification of hydroquinone and phenol saline wastewater, must be considered.

P 20

Acute toxicity of fenoxaprop-p-ethyl formulations on mosquito fish (Gambusia affinis) and Vibrio fischeri

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Acute toxicity of pesticides is considered to be of primary importance in our efforts to assess ecological risk. In spite of the obvious significance of chronic toxicity data for ecological risk assessment, either due to lack of such data or due to the nature of pesticides under study acute toxicity data are being used (Palma et al. 2004)). Yet, in most cases, in spite of the presence of compound mixtures, pollution due to pesticides is being handled on the basis of critical toxicological values (ECs, NOECs) for each single compound. This often results to a non realistic assessment, particularly if the scope of the study is far more than a preliminary estimation. The problem has been recently recognized by a lot of authors (Altenburger et al. 2000, 2003, 2004) and various models are suggested (Belden et al. 2007) aiming a good predictability. Recently, aiming to a better crop selectivity as well as a broader weed spectrum , herbicide mixtures have been developed. These formulations contain either an antidote (a safener for better crop selectivity) or/and other compounds (for a better weed spectrum). Fenoxaprop-p-ethyl (a folar acting arylphenoxypropionic acid) is a striking example of this strategy. The compound is currently being applied formulated either with the antidote mephenpyr-diethyl or/and iodosulfuron (a sulfonylurea controlling broadleave weeds in cereals). Due to fenoxaprop use in rice and wheat as well as iodosulfuron use in wheat and corn the ecological significance of these formulations (surface waters contamination through runoff) is obvious. Acute toxicity data on aquatic fauna for these formulations are only limited to the single herbicidal compounds. The aim of this study was to assess the acute toxicity of fenoxaprop- p -ethyl (55 g a.i./L) + fenchlorazol-ethyl(30 g a.i./L,) (FP + FCZ) vs. fenoxaprop- p -ethyl (64 g a.i./L) + mefenpyr-diethyl (24 g a.i./L) + iodosulfuron-methyl (8 g a.i./L) (FP + MFP + IOD) as well as iodosulfuron-methyl (100 g a.i./L) + mefenpyr-diethyl (300 g a.i./L) (IOD + MFP) upon Gambusia affinis and Vibrio fischeri. The teleost viviparous fish Gambusia affinis is found in abundance in ponds and smalls streams in the region of Thrace in Northern Greece and is often exposed to pesticides either directly or indirectly through leaching and runoff. Mosquitofish is, therefore, considered not only as a valuable bioindicator for monitoring of xenobiotics in the region but also as a test aquatic organism for pesticide toxicity assessment (Rendon-von Osten et al. 2005). Vibrio fischeri was chosen as the part of the easy to handle and well standardized Microtox test according to SDI procedures.

Samples of naturally occurring population of Gambusia affinis were transferred to the laboratory. Within 21 days natural water was gradually substituted by moderately hard tap water fully dechlorinated through aeration. Fish were finally acclimated for a period of seven days under the conditions of the experiment. The average wet weight and total length of fish used in the experiments were 1.0 ± 0.4 g and 3 ± 0.5 cm for males and females respectively. Acute toxicity tests (3 tests for each formulation and exposure period)) were conducted according to standard procedures (OECD, 1992). Exposure period was 24 and 48 hours. Mortality was recorded at the end of each 24 h period. The static test procedure (non renewal) was applied. Water quality parameters such as pH, temperature, dissolved oxygen and specific conductivity of the test solution and controls were measured and recorded daily during the testing period . At the beginning of the test, 10 adults (5 males + 5 females) were randomly distributed among the test chambers. LC50 values were determined by using the R language and environment for statistical computing.The add-on package

drc (Ritz and Streibig, 2005) was used to fit the four-parameter Weibull model. The Weibull model was selected based on Akaike´s information criteria (AIC) from the models logistic, Gompertz, Brain-Cousens.
For each formulation and exposure time means of the three experiments are presented since the application of the Box-Cox transformation method (Streibing et al. 1998) revealed homogeneity of the response curves (the optimal λ parameter was not significantly different from 1.00).
Acute toxicity tests on Vibrio fischeri (3 tests for each formulation and exposure period) were conducted in a Microtox Model 500 analyzer according to standard SDI procedures as prescribed for the basic 45% standard test for 5 and 15 minutes.

Formulation	Exposure period	Slope estimate	Standard error	LC50 (mg/L)	Confidence interval
IOD+MFP	24 h	0.95	0.13	1.10	0.98 - 1.3
IOD+MFP	48 h	0.64	0.10	1.09	0.97 - 1.28
FP+IOD+MFP	24 h	1.025	0.21	0.48	0.37 - 0.63
FP+IOD+MFP	48 h	1.029	0.22	0.50	0.33 - 0.72
FP+FCZ	24 h	0.95	0.07	0.73	0.67 - 0.90
FP+FCZ	48 h	0.40	0.11	0.58	0.53 - 0.65

Table 1. Estimated LC_{50s} for fenoxaprop- p -ethyl + fenchlorazol-ethyl (FP+FCZ) , fenoxaprop- p -ethyl + mefenpyr-diethyl + iodosulfuron-methyl (FP+IOD+MFP) and iodosulfuron-methyl + mefenpyr-diethyl (IOD+MFP) on *Gambusia affinis* after 24 and 48 hours of exposure

Concerning acute toxicity on Gambusia affinis (Table 1) after 24 hours of exposure FP+FCZ seems to take an intermediary position among the other two formulations. IOD+MFP is the least toxic one. However, even if toxicity data of the single ingredients upon Gambusia affinis are not available, fish toxicity of the single ingredients is much lower. According to Bayer, iodosulfuron has a 96 h LC50 of 117 mg/L on Cyprinus caprio, while the LC50 of MFP on rainbow trout is 2.4 mg/L (Bayer safety data sheets, 2004, 2008). FP+IOD+MFP proved to be much more toxic than IOD+MFP in both exposure times and more toxic than FP+FCZ after 24 h of exposure. A potentation of the toxicity of IOD+MFP through the addition of FP could be under consideration. It seems that in all three formulations a period of 24 hours of exposure is enough to assess acute toxicity.

Formulation	Exposure period	EC50 (mg/L)	limits	Slope
IOD+MFP	5 min	0.835	0.66 - 1.05	0.85
IOD+MFP	15 min	0.860	0.77 - 1.25	1.057
FP+IOD+MFP	5 min	0.118	0.092 - 0.15	0.74
FP+IOD+MFP	15 min	0.14	0.1 - 0.22	0.75
FP+FCZ	5 min	0.046	0.038 - 0.055	0.93
FP+FCZ	15 min	0.044	0.033 - 0.058	0.81

Table 2. Estimated EC_{50s} for fenoxaprop- p -ethyl + fenchlorazol-ethyl (FP+FCZ) , fenoxaprop- p -ethyl + mefenpyr-diethyl + iodosulfuron-methyl (FP+IOD+MFP) and iodosulfuron-methyl + mefenpyr-diethyl (IOD+MFP) on *Vibrio fischeri* after 5 and 15 minutes of exposure

Table 2 shows the acute toxicity of the formulations upon Vibrio fischeri. IOD+MFP proved also in this case to be the least toxic formulation. On the other hand, Vibrio fischeri seemed to be extremely sensitive to FP+FCZ with an LC50 ranging from 0.033 - 0.058 mg/L. FP is generally considered fish toxic (LC50 96 h for rainbow trout 0.39 mg/L, Bayer safety data sheet, 2007), while for FCZ no data exist. Also in this case FP+IOD+MFP proved to be more toxic than IOD+MFP which was the least toxic one. Comparing the two test species, Vibrio fischeri was much more sensitive than Gambusia affinis to the formulations containing FP (FP+IOD+MFP and FP+FCZ).

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Adsorption of imazethapyr herbicide onto montmorillonite as affected by Cu and Cu-humic acids

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Adsorption of imazethapyr, an imidazolinone herbicide, was measured from dilute suspensions onto montmorillonite and complexes of montmorillonite with Cu and humic acid.
Imazethapyr adsorption was greater for the Cu-montmorillonite and Cu-humic acid-montmorillonite complexes than by montmorillonite. Isotherm shape was similar for montmorillonite, Cu-montmorillonite and Cu-humic acid-montmorillonite.
Adsorption of imazethapyr varied with pH for montmorillonite and for Cu-humic acid-montmorillonite complex. The pH had no apparent effect on imazethapyr adsorption to the Cu-montmorillonite complex. This might be due to imazethapyr retention at high pH by Cu ions linked to the clay.
Imazethapyr adsorption kinetics was faster for Cu-montmorillonite (15 min) and Cu-humic acid-montmorillonite (30 min) than for montmorillonite (240 min). These results suggest that Cu and humic acid complexes with montmorillonite might be used to detoxify water contaminated with imazethapyr.

P 86

Black Chemistry: on the travel of dioxins and furans from Brazil to Germany in the late 1990´s

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PCDD/Fs and other persistent organic pollutants (POPs) emitted from historical sources can persist in soil/sediments and waste reservoirs for decades or even centuries. Therefore problems associated with PCDD/Fs should be considered to a large extent a legacy issue and contaminated sites are likely to remain a problem not only now, but also in the future. One important issue to consider are the pathways through which PCDD/Fs and POPs enter the food web. Special attention must be paid to how theses compounds might be introduced into the industrial food products. Here, In addition to entering the food chain through the standard mechanisms of bioaccumulation and biomagnification, of environmental PCDD/Fs and other POPs can enter the food chain through a range of “short cuts” directly contaminating feeds and foods. One such documented case occurred in 1997 when dioxin concentrations in milk from different farms in Baden-Württemberg (SW Germany) suddenly increased.

After years of slowly declining values, the mean dioxin content in milk rose from 0.62 pg I-TEQ/g fat to 1.41 pg I-TEQ/g fat within half a year. The peak concentration in this sample was almost 8 pg I-TEQ/g fat. Soon, similar concentrations in milk and dairy products were reported from several regions of Germany and other European Union member states. The first step was to find the contamination source, which turned out to be citrus pulp pellets (CPP) imported from Brazil used for cattle feed. Citrus pulp, a former residue of orange juice production, nowadays is added in the form of pellets to feed for ruminants due to its high energy content. Dioxin concentrations in the contaminated CPP samples ranged from 5 to 10 ng I-TEQ/kg. The PCDD/F contamination source for the CPP was the 2% lime added to the wet peels corresponding to about 7-8% of the dry CPP weight. Following the ban on CPP exports, Brazilian CPP producers lost at least USD 40 million, according to the Brazilian Association of Citrus Exporters. 40 thousand tons of contaminated pulps stored in the Netherlands were destroyed at a cost of USD 6 million. This embargo was lifted cancelled by the European authorities only after the source of the contamination was eliminated, and the Brazilian Ministry of Agriculture had implemented a dioxin regulation for CCP and lime with the limit of 0.5 ng I-TEQ/kg complying with the European standard (European Directive 98/60/EC) and had established a continuous monitoring program of PCDD/Fs in CPP and lime. We believe that the contaminated lime was purchased from one supplier using lime from a contaminated site. However, details about the origin of the PCDD/Fs in the contaminated lime were not revealed. This work further investigates the contamination origin and describes the link between lime milk from the dumpsite of a chlorine/organochlorine industry and the contaminated hydrated lime.

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P 87

Organochlorine pesticides in Nile tilapia (Oreochromis niloticus) from fish farms in Brazil

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Organochlorine compounds are one very important class of persistent organic pollutants, that due to bioaccumulation and toxicity, derived also from its chemical stability, have long biological half lifes that is governed by lipophilicity. Farmed fish can be exposed to persistent organic pollutants - such as organochlorine pesticides (OCP) - via feed. The concentrations of OCP were determined in fish samples collected from different fish farms in Brazil. Muscle samples of Nile Tilapia of two age classes (juvenile -J with 60-90g and adult - A with 500-700g), from four fish farms (2 intensive and 2 super intensive culture system), were analyzed by means of high resolution gas chromatography coupled to mass spectrometry (HRGC-HRMS). DDT and related compounds were the predominant organohalogenated contaminants in all four fish farms, with the p,p-DDE contributing to more than 69% to the ΣDDTs, followed by p,p-DDD with more than 16%. The means concentrations of ΣDDTs, hexachlorocyclohexane (HCH) isomers, hexachlorobenzene (HCB), chlordanes and others seven pesticides (aldrin, dieldrin, endrin, endosulfan-I and II, methoxychlor and mirex) were 277 pg.g-1, 70.1 pg.g-1, 139 pg.g-1, 32.9 pg.g-1 and 159 pg.g-1, respectively. Comparing the levels of organochlorines and lipid content in super intensive and intensive fish farms, we found that super intensive fish farms showed higher lipid contents and consequently higher levels of some pesticides, which were 59% of HCH, 58% of HCB, 69% of ΣDDTs and 65% of aldrin and others for super intensive fish farms, exceed chlordanes that were 56 % higher in intensive fish farms. No correlation between length/weight (and therefore age) of fish and DDE concentration were observed, probably means that young tilapia may be susceptible to pesticide stress but with time develop physiological strategies to compensate it. However, all pesticides levels did not exceed the Maximum Residue Limit (MRL) recommended by FAO/WHO.

P 88

Polychlorinated biphenyls (PCB) in Nile tilapia (Oreochromis niloticus) from fish farms in Brazil

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Tilapia is the most popular freshwater fish farmed in Brazil, being the second most important group of fish feed in the world. Farmed fish can be exposed through alimentary intake to persistent organic contaminants, such as polychlorinated biphenyls, due to a diet based in fish oil and fish meal. The concentrations and congener's pattern of the PCBs were determined in samples collected from different fish farms in Brazil, in different locations. 80 muscle samples of Tilapia (Oreochromis niloticus) of two age classes: juveniles weighted 50-80g and adults weighted 500-700g, from four fish farms (2 intensive and 2 super intensive culture system) were analyzed. The results indicated that the PCB mean concentrations in fish and fish feed samples ranged from 93.3 to 364.9 and from 69.3 to 2310.4 pg g-1 fresh wt basis, respectively. PCB were analyzed into three categories as follow: indicator PCB (90.1% of total samples, majority found at super intensive systems), non-ortho (were present in 0.5% of total samples) and mono-ortho (9.4% of total samples). The major levels of PCB congeners found were (wet weight) in the following rank 153 (89.9 pg/g) > 28 (73.0 pg/g) > 52 (71.2 pg/g) > 138 (58.1 pg/g) > 101 (54.4 pg/g) > 180 (33.7 pg/g), with a ratio PCB 153/sum PCB of 0.16. Of the 40 fish samples analyzed, all contained all PCB, except PCB 126 and 169 which were found just in intensive fish farms. PCB 77 was found just in super intensive fish farm 1, indicating a possible local contamination. Low levels of PCB were found in fish feed analyzed and can explain the low PCB content in fish flesh, in spite of the intensive culture conditions. The levels did not exceed the Maximum Residue Limit (MRL) recommended by FAO/WHO.

Poster Session 2 - Environmental Health, Resources & Waste and Green Products

P 21

Evaluation of the possible human dietary exposure to toxic chemicals via the consumption of wild medicinal plants

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A broad survey of a number of persistent halogenated organic pesticide and toxics heavy metals was carried out in a large number of wild medicinal herbs known to grow wildy in North Africa wilderness and claimed by general public to be of therapeutic importance.
The herbs subjected to our study were those found to grow rather close to the agriculture areas where the uncontrolled usage of halogenated chemicals is not unusual practice, also where some near by industrial activities could be blamed for halogenated compound and heavy metal pollution. Representative samples of the herbs from different families were carefully colleted from Libyan wilderness then analyzed for their contents of the halogenated organic and the metals pollutants.
The presence and the concentration pattern of the determined chemicals were studied regarding to the plants, families and the different environmental aspects. Interestingly the results reveled that different families seem to show different concentration trends of the pollutants.
Hair, blood and nail samples of the selected members of the public were extensively investigated for the suspected pollutants.

P 22

Determination of some heavy metals, chlorinated hydrocarbons and PAHs in the salt and water from the Al Jabboul saline of Syria

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Summary: After several scientific studies on different specimens of the water and salt produced from Al Jabboul Saline in 1993, 1994, 1995, 1996 successively, and in 2006 and 2007 in particular, in order to specify some organic and inorganic contaminants therein; it has been demonstrated that concentrations of some heavy metals, such as lead (Pb), cadmium (Cd) and arsenic (As), expressed in a particle per million (ppm), exceeded the internationally permissible concentrations in food stuffs for each metal. Concentration of aluminum (Al) reached 30.09 ppm in 1995, and that of sulfur reached 6860 ppm in 1994. Concentrations of some chlorinated organic compounds have drastically increased in the last ten years to 75 - 100 times, but concentration of some poly-aromatic hydrocarbons (PAHs) did not exceed 1.4 ppm.

From the above, we may conclude that there is a distinct risk on consumers of Al-Jabboul Saline salt in the next decades due to the increasing accumulation of contaminants of other weekly household diet (WHO) in human body, in addition to the contaminants of this salt.

Introduction: After the constant increase of all types of contaminants, particularly in the last three decades, it has become an obligation for the developed and developing countries to concentrate their main scientific concerns on improving food stuff production and providing them to the consumers free from all contaminants, which have become a heavy burden and inevitable danger on human life. To investigate the presence of the various contaminants in some food stuffs, researchers calculated the quantity of heavy metals permissible in the weekly nutrition meals, for each of males and males, and called it "the weekly household diet (WHO)"1)

Since table salt is considered one of the different food substances and is greatly important for human beings, it has to be supplied to the people free from the dangerous contaminants. In case of presence of such contaminants, their concentrations should not exceed the maximum limit internationally permitted, as shown in table No. (1), as per (Codex standard 150-1985).

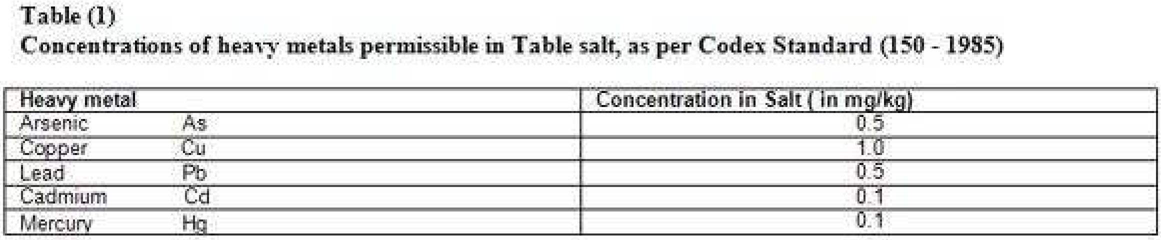


Table (1)
Concentrations of heavy metals permissible in Table salt, as per Codex Standard (150 - 1985)

Heavy metal		Concentration in Salt (in mg/kg)
Arsenic	As	0.5
Copper	Cu	1.0
Lead	Pb	0.5
Cadmium	Cd	0.1
Mercury	Hg	0.1

[1]

The target and important of this research is in performing the necessary study on the water and salt of this saline to achieve scientific results that clarify to us Al Jabboul salt level of contamination with various contaminants, and to act to limit it in case of its presence.

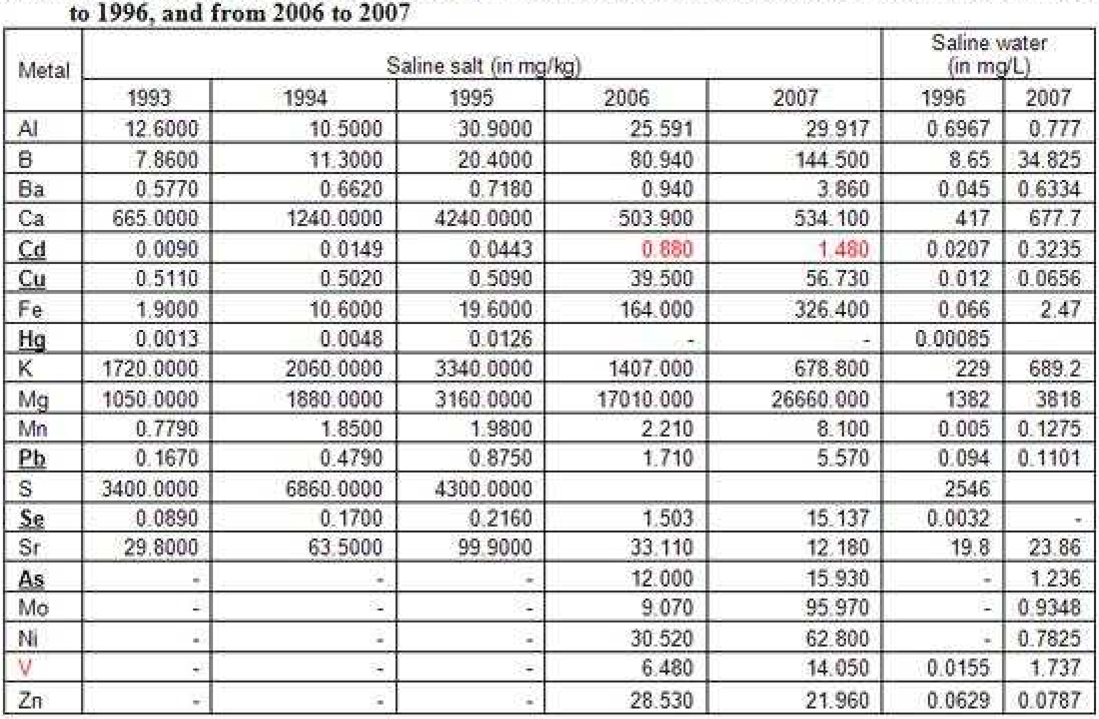
Material and methods: We have attempted, in this research, to specify concentrations of three sets of organic compounds contaminating Al Jabboul, which are:

a - Some light and heavy metals.
b - Some chlorinated organic compounds.
c - Some poly-aromatic hydrocarbon (PAHs) compounds.

a - Determination of light and heavy metals:

In this regards, we have adopted the method of inductive coupled plasma spectroscopy (ICP) 2)
We prepared diluted specimens to 40 times, and took 10 micro liters of which to inject it in the ICP-paratus. Table No. (2) gives us an idea about the quality and concentrations of Al Jabboul Saline heavy and light metals using ICP - spectroscopy.

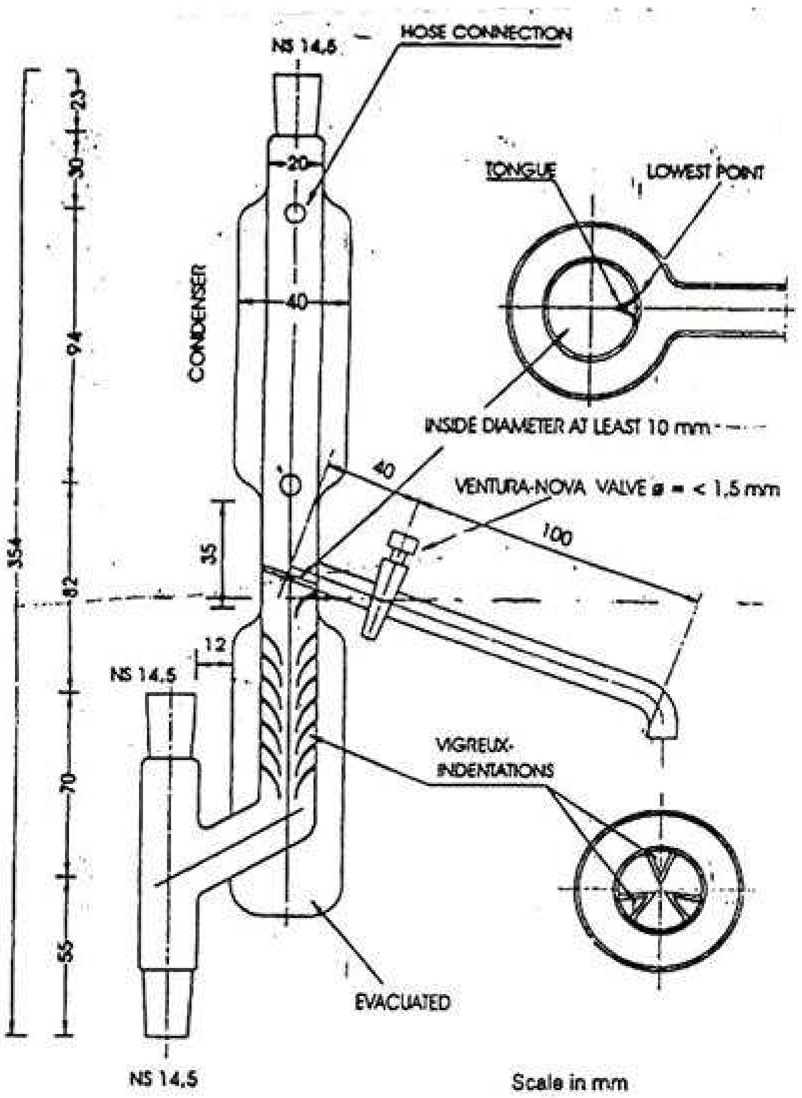
Table (2): Concentrations of some heavy and light metals present in Al Jabboul salt and water from 1993 to 1996, and from 2006 to 2007



Metal	Saline salt (in mg/kg)					Saline water (in mg/L)	
	1993	1994	1995	2006	2007	1996	2007
Al	12.6000	10.5000	30.9000	25.591	29.917	0.6967	0.777
B	7.8600	11.3000	20.4000	80.940	144.500	8.65	34.825
Ba	0.5770	0.6620	0.7180	0.940	3.860	0.045	0.6334
Ca	665.0000	1240.0000	4240.0000	503.900	534.100	417	677.7
Cd	0.0090	0.0149	0.0443	0.880	1.480	0.0207	0.3235
Cu	0.5110	0.5020	0.5090	39.500	56.730	0.012	0.0656
Fe	1.9000	10.6000	19.6000	164.000	326.400	0.066	2.47
Hg	0.0013	0.0048	0.0126	-	-	0.00085	
K	1720.0000	2060.0000	3340.0000	1407.000	678.800	229	689.2
Mg	1050.0000	1880.0000	3160.0000	17010.000	26660.000	1382	3818
Mn	0.7790	1.8500	1.9800	2.210	8.100	0.005	0.1275
Pb	0.1670	0.4790	0.8750	1.710	5.570	0.094	0.1101
S	3400.0000	6860.0000	4300.0000			2546	
Se	0.0890	0.1700	0.2160	1.503	15.137	0.0032	-
Sr	29.8000	63.5000	99.9000	33.110	12.180	19.8	23.86
As	-	-	-	12.000	15.930	-	1.236
Mo	-	-	-	9.070	95.970	-	0.9348
Ni	-	-	-	30.520	62.800	-	0.7825
V	-	-	-	6.480	14.050	0.0155	1.737
Zn	-	-	-	28.530	21.960	0.0629	0.0787

[2]

b - Determination of chlorinated organic compounds concentrations: <br 10 liters of Al Jabboul Saline water have been extracted by means of n-Hexan organic dissolvent by adding 50 ml of the organic dissolvent to each 500 ml of Al Jabboul Saline water . We gathered the organic layers in a volume flask and evaporated the organic solution by means of the rotating evaporation apparatus till the volume became 5 ml. We continued the evaporation process by means of a modified evaporation apparatus used for chlorinated organic compounds designed by Kuderna Danish till the volume became 2 ml, as illustrated in figure No. 1. We purified the extracted specimen by high potency liquid chromatography (HPLC) 3). To specify the quality and concentrations of herbicides and insecticides in the chosen specimen, we prepared 7 diluted specimens of the standardized solution (I), under the following form: 4 pg/µl, 5 pg/µl, 12.5 pg/µl, 25 pg/µl, 50 pg/µl, 100 pg/µl, 200 pg/µl. To specify the quality and concentrations of biphenyl compounds (PCB)4)3 in the selected specimen, we also prepared 7 diluted standard specimens, as above from the standardized solution No. (II).We injected 2 micro liters of each diluted standardized solutions specimen in the capillary gas chromatographic system (GC/ECD) and got a standardized curve through which we were able to specify concentrations of insecticides,herbicides and biphenyl compounds after injecting 2 micro liters of the research selected specimen in the same apparatus.



– Kuderna -Danish

[3]

Figure No 1

Specifications of the GC/ECD- apparatus used were as follows: Quartz capillary with solid phase DB 1701, l=3 m, i.d.=0,32 mm, film=0,25 u and DB 5, l=30 m, i.d.=0,32 mm, film=0,25 um. Carrier gas: H2 (4 ml/min., linear gas velocity: 82 cm/sec at 25 C°). Temperature programme, for example: 60 C° to 120 C° with 8 C°/min . after 3 min. up to 260 C° with 5 C°/min., then up to 280 C° with 10 C°/min. Sample application: 1 ul, split/splitless, Injector temperature: 280 C°, ECD temperature 300 C°, make-up gas N$_2$, 50 ml/min.

The standardized solution No. (I) consists of: HCB, β-HCH, o,p-DDT, p,p-DDT, p,p-DDE, o,p-DDD, P,p-DDD, Dieldrin , Chlordan, Endrin, Heptachlor, t-Heptachlorperoxyd, β-Endosulfan .

The standardized solution No. (II) consists of: 2,4,4$^{/}$ -Trichlorbiphenyl, 2,2,5,5$^{/}$ -Tetrachlorbiphenyl, 2,2$^{/}$,4,5,5$^{/}$ -Pentachlorbiphenyl, 2,2$^{/}$,3,4,4$^{/}$,5$^{/}$ -Hexachlorbiphenyl, 2,2$^{/}$,4,4$^{/}$,5,5$^{/}$ -Hexachlorbiphenyl, 2,2$^{/}$,3,4,4$^{/}$,5,5$^{/}$ -Hepta chlorbiphenyl

To specify the chlorinated organic compounds in the solid salt specimens, (1) g of each salt specimen was weighed and diluted in 250 ml of distilled water. 25 ml of the organic dissolvent n-Hexan was added to it and was treated in the same manner of treating the water specimens.

Tables No. (3) and No. (4) give us an idea about concentrations of some chlorinated organic compounds available in each of Al Jabboul Saline water and salt.

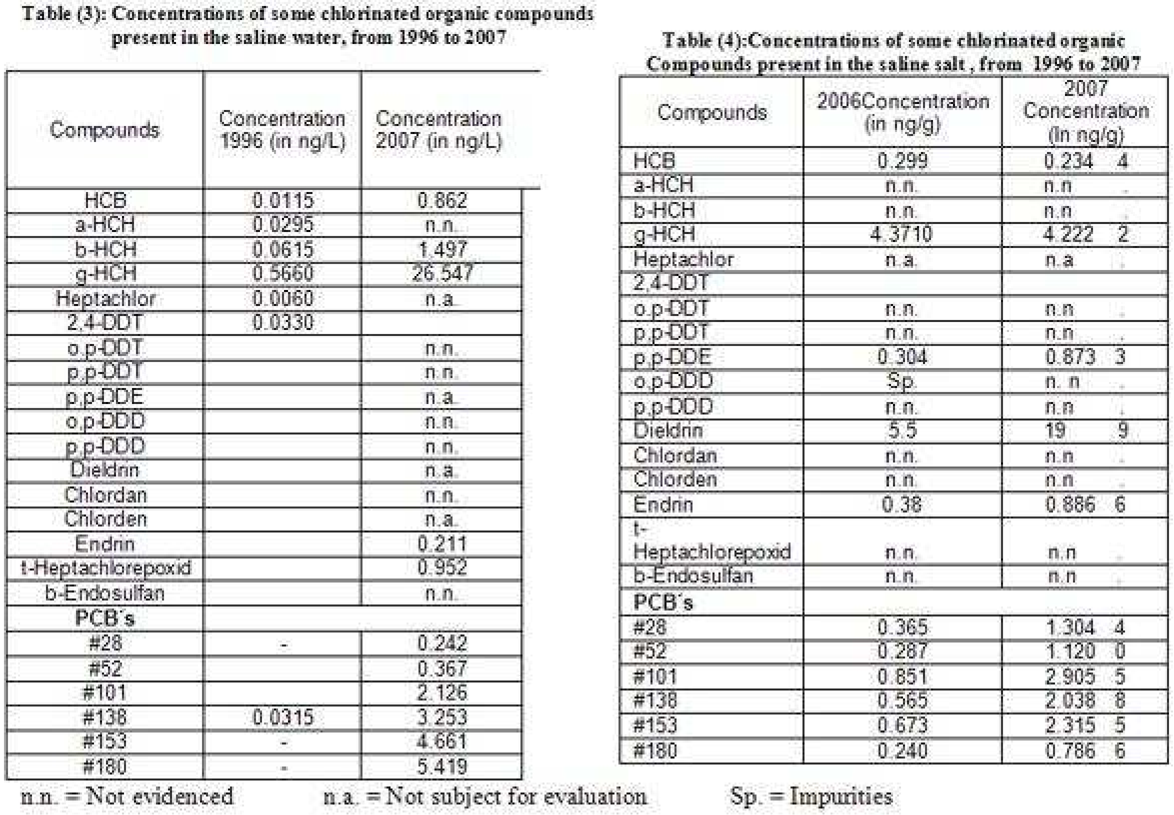


Table (3): Concentrations of some chlorinated organic compounds present in the saline water, from 1996 to 2007

Compounds	Concentration 1996 (in ng/L)	Concentration 2007 (in ng/L)
HCB	0.0115	0.862
a-HCH	0.0295	n.n.
b-HCH	0.0615	1.497
g-HCH	0.5660	26.547
Heptachlor	0.0060	n.a.
2,4-DDT	0.0330	
o,p-DDT		n.n.
p,p-DDT		n.n.
p,p-DDE		n.a.
o,p-DDD		n.n.
p,p-DDD		n.n.
Dieldrin		n.a.
Chlordan		n.n.
Chlorden		n.a.
Endrin		0.211
t-Heptachlorepoxid		0.952
b-Endosulfan		n.n.
PCB´s		
#28	-	0.242
#52		0.367
#101		2.126
#138	0.0315	3.253
#153	-	4.661
#180	-	5.419

Table (4):Concentrations of some chlorinated organic Compounds present in the saline salt , from 1996 to 2007

Compounds	2006Concentration (in ng/g)	2007 Concentration (In ng/g)
HCB	0.299	0.234 4
a-HCH	n.n.	n.n .
b-HCH	n.n.	n.n .
g-HCH	4.3710	4.222 2
Heptachlor	n.a.	n.a .
2,4-DDT		
o,p-DDT	n.n.	n.n .
p,p-DDT	n.n.	n.n .
p,p-DDE	0.304	0.873 3
o,p-DDD	Sp.	n. n .
p,p-DDD	n.n.	n.n .
Dieldrin	5.5	19 9
Chlordan	n.n.	n.n .
Chlorden	n.n.	n.n .
Endrin	0.38	0.886 6
t-Heptachlorepoxid	n.n.	n.n .
b-Endosulfan	n.n.	n.n .
PCB´s		
#28	0.365	1.304 4
#52	0.287	1.120 0
#101	0.851	2.905 5
#138	0.565	2.038 8
#153	0.673	2.315 5
#180	0.240	0.786 6

n.n. = Not evidenced n.a. = Not subject for evaluation Sp. = Impurities

[4]

c - Identification of the poly-aromatic hydrocarbon compounds (PAHs):

To achieve this purpose we diluted (1) gram of each solid salt specimen in 250 of distilled water and transferred the solution to a 750 ml volume flask. We added 5 ml of concentrated sulfur acid (H_2SO_4) and 15 ml of (CH_2CL_2) and stirred the mixture with the magnetic stirrer for one hour. After the process of separating the organic layer from the water layer, we collected the organic layers, cleared them from water residuals by adding (1) g of dehydrate sodium sulfate and left the mixture for about 30 minutes. After the filtration process, we transferred the organic solution to a small volume flask and evaporated it with the rotating evaporation apparatus till the volume became 2 ml. We transported the evaporated specimen on a small 8 cm long chromatographic separation column with a diameter of a cm, filled with 2 g of siliceous gelatin, with a grain diameter of 40 microns. Then, we added 10 ml of the movable phase mixture, which consists of di-chloride methane and cyclohexane (1:1).We gathered the extraction in a small volume flask and evaporated it by a current of N_2 gas till it got dry, then we added 500 micro liter of the organic dissolvent (acetonitrile) to the dry specimen. To specify the concentrations of PAHs compounds in the water research specimens, we prepared 7 standard diluted solutions of the diluted standardized solutions specimen in the (GC/ MS) apparatus, and consequently we got a standard curve through which we managed to specify the PAHs [5-6])compounds .

Standardized solution No. (III) consists of: Acenaphthene, Acenaphthylene, Antracene, Benzo(a)anthracene, Benzo(b)fluoranthene, Benzo(k)fluoanthene, Benzo(g,h,i)porylene, Benzo(a)pyrene, Chrysene, Diben(a,h) Anthracene, Fluoranthene, Fluorene, Indeno(1,2,3,c,d)pyrene, Naphthalene, Phenanthrene, Pyrene .

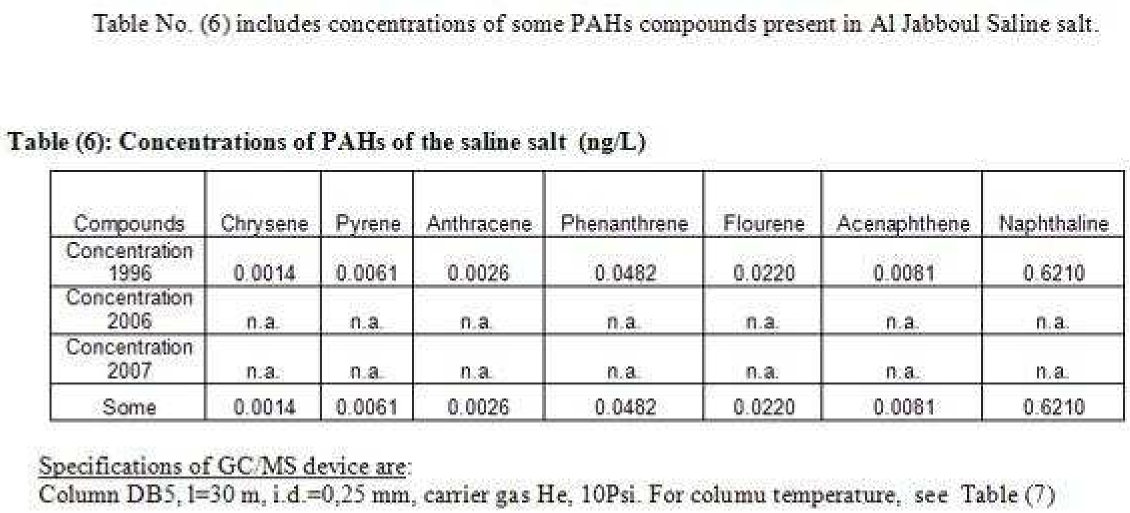


Table No. (6) includes concentrations of some PAHs compounds present in Al Jabboul Saline salt.

Table (6): Concentrations of PAHs of the saline salt (ng/L)

Compounds	Chrysene	Pyrene	Anthracene	Phenanthrene	Flourene	Acenaphthene	Naphthaline
Concentration 1996	0.0014	0.0061	0.0026	0.0482	0.0220	0.0081	0.6210
Concentration 2006	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Concentration 2007	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Some	0.0014	0.0061	0.0026	0.0482	0.0220	0.0081	0.6210

Specifications of GC/MS device are:
Column DB5, l=30 m, i.d.=0,25 mm, carrier gas He, 10Psi. For columu temperature, see Table (7)

[5]

Results and discussion: A through look on Table No. (2), which includes concentrations of some heavy and light metals present in Al Jabboul Saline waters and salt, leads us to the following conclusions:

1- The increase of a large number of heavy and light metals within the last ten years in Al Jabboul salt, such as aluminum (Al), calcium (Ca), copper (Cu), iron (Fe), Lead (Pb), Mercury (Hg), selenium (Se), and arsenic (As), is related to the increase in pouring the discharge water of the twelve tributaries in the saline body.

2- The highest concentration of heavy metals elements in Al Jabboul Saline is lead concentration, which was 5.570 ppm, and arsenic concentration which reached 5.930 ppm in 2007.

3- Based on person average daily consumption of 20 g of Al Jabboul salt, we conclude that each person consumes 0.111 mg/day, which equals 0.7798 mg/week of lead, 0.3186 mg/day, which equals 2.2302 mg/week of arsenic, 0.2072 mg/week of cadmium, and 0.0025 mg/week of mercury.

4- Values included in Table No. (3) show that concentration of each HCB, b-HCH and -HCH of Al Jabboul salt has doubled from 5 to 75 times, and concentration of PCBs compound No. (138) has doubled 100 times in the years 2006-2007. 138

5- Values in Table No. (4) indicate that concentrations of PCBs compounds Nos. 28, 52, 101, 138, 153, 180, , have doubled 3-4 times in Al Jabboul salt in the years 2006-2007.

From the above, we may conclude that there is a distinct risk on Al Jabboul salt consumers in the next years. This risk does not only lie in accumulation of this salt inside the live body, but also in accumulation of other contaminants available in the other various nutrition materials.

Acknowledgement: I thank Prof. Dr. Dr. H. Parlar, director of the Institute of analyzing technical chemistry and food chemical Technology of the Technical University of Munich, Germany, for his assistance in solving various Problems.

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P 23

Identification, characterization and impact assessment of a park solar photovoltaic (Murcia, Spain)

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The Region of Murcia (Spain), wants to take his appeal and plans to install a solar photovoltaic solar Park respecting the environment in the population of Blanca. The purpose of this study is to identify, assess and analyse the space physical, biological and socioeconomic Project. To identify, characterize and assess the nature and magnitude of the effects caused by the construction of the project and its operation will determine the feasibility of it.

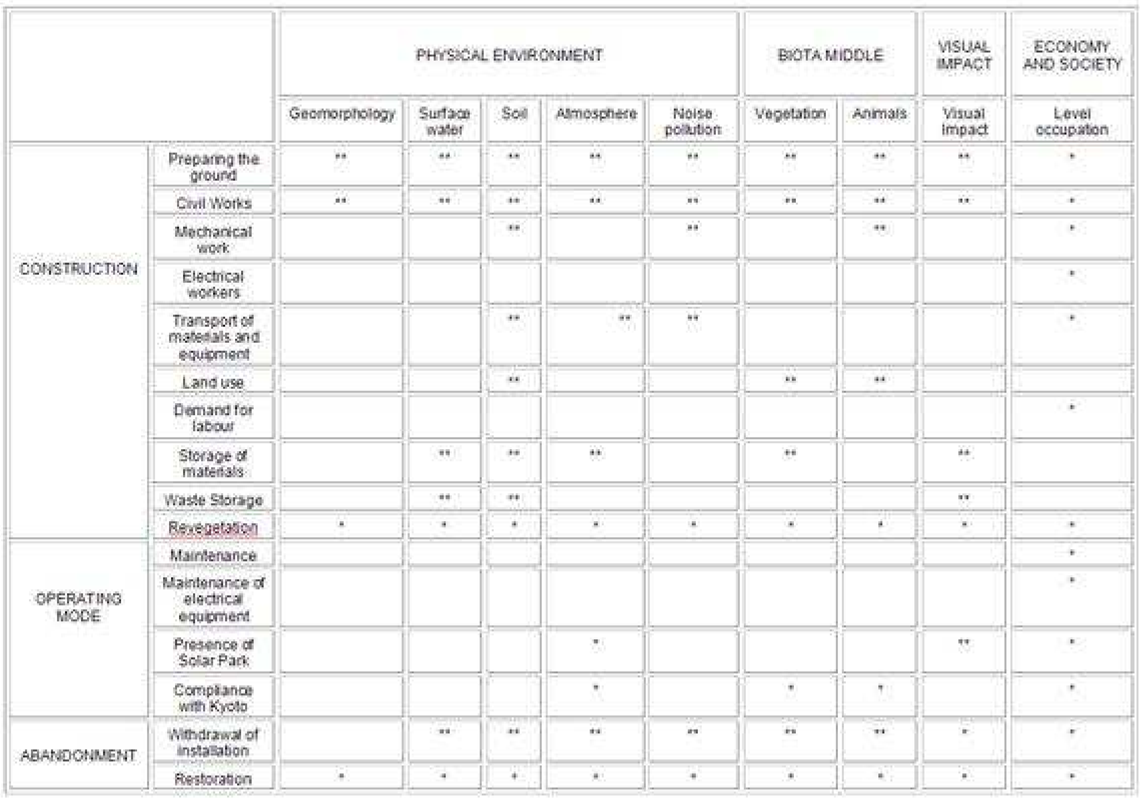
Introduction: In a park Solar Photovoltaic solar radiation is used to generate electricity by seizing the physical properties of the cells that make semiconductor plates. The photovoltaic solar energy is renewable, inexhaustible, clean and respectful of the environment. This energy contributes to the reduction of emissions of CO2 and other greenhouse gases, helping to comply with the resolutions adopted at the Kyoto Protocol. The Region of Murcia (Spain), wants to take his appeal solar, using the latest technologies, with the criterion of maximum respect for the environment and the environment. The Draft Photovoltaic Solar Park is located in the town of Blanca (Murcia, Spain). Occupies an area of 3 hectares, with an output of 1.5 MW and is the operator Iberdrola S.A.U. This is an area of tree crops irrigated with any condition on holding prominent on the landscape. The hydrology of the area was not affected in the absence of drainage channels or formations within the plot. There is no space affects protected natural or environmentally sensitive area. Given the topographical features of the terrain, its extension and the unavailability of resources (water and natural gas), make the best alternative, environmentally and technically, is the introduction of a Central Solar photovoltaic panels fixed.

Methodology: The methodology is based on the work methodology of Evaluation Environmental Impact and has studied the following sections: Description of the project; Consideration of alternatives; environmental Inventory (physical, biotic and archaeological) and Impact Assessment, characterization and objective assessment of the project.

Results and discussion: We appreciate and characterize the environmental impacts on each element in the construction phase, the phase of operation and during the abandonment of the installation.

- Phase identification (Table 1): detailing the changes that the various actions of the Project will generate in geomorphology, surface water and groundwater, soil, vegetation, wildlife, noise, air, and socioeconomic landscape, identifying the environmental impacts it generates development Project. They are used in a series of tables facing the elements of the physical environment, biological and social action inherent in the project.
- Phase characterization and valuation (Tables 2 and 3): alterations are characterized both in the construction phase, as in the operating and abandonment. This characterization is done through criteria valuations impact (characteristic, type of action, duration, etc.). That are reflected in a range of levels of impact (consistent, moderate and severe critic), which facilitates use the results obtained in decision-making.

TABLE 1.- IDENTIFICATION OF PROJECT ACTIONS THAT CAN CAUSE IMPACTS

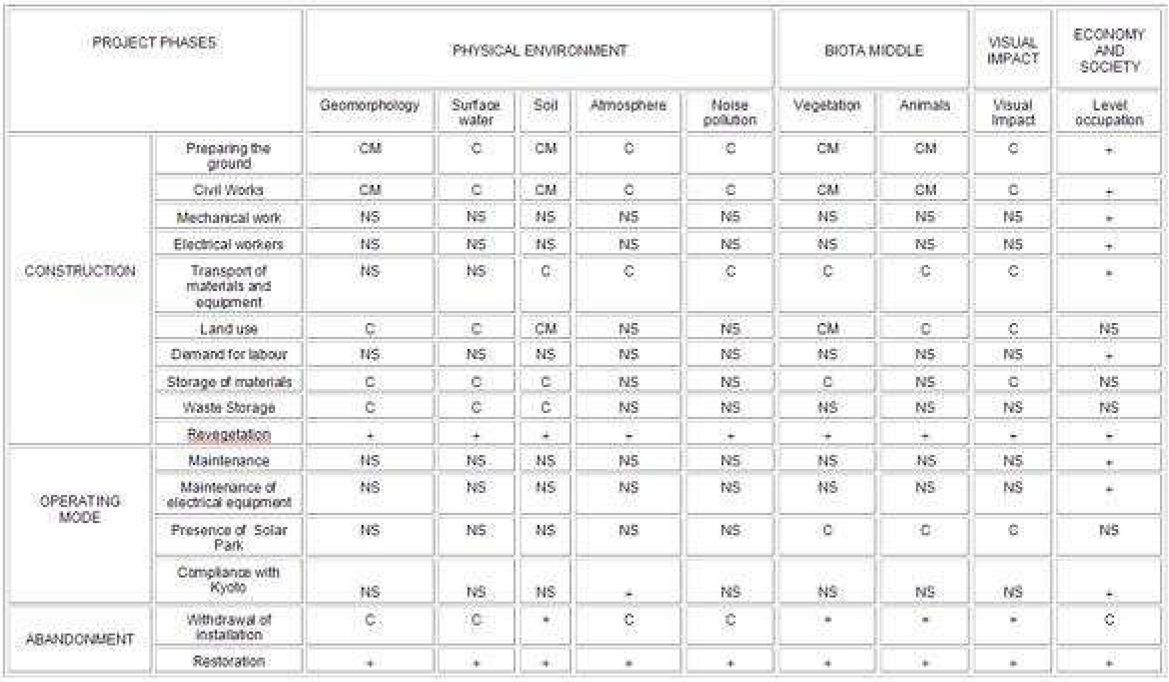


		PHYSICAL ENVIRONMENT					BIOTA MIDDLE		VISUAL IMPACT	ECONOMY AND SOCIETY
		Geomorphology	Surface water	Soil	Atmosphere	Noise pollution	Vegetation	Animals	Visual Impact	Level occupation
CONSTRUCTION	Preparing the ground	**	**	**	**	**	**	**	**	*
	Civil Works	**	**	**	**	**	**	**	**	*
	Mechanical work			**		**		**		*
	Electrical workers									*
	Transport of materials and equipment			**	**	**				*
	Land use			**			**	**		
	Demand for labour									*
	Storage of materials		**	**	**		**		**	
	Waste Storage		**	**					**	
	Revegetation	*	*	*	*	*	*	*	*	*
OPERATING MODE	Maintenance									*
	Maintenance of electrical equipment									*
	Presence of Solar Park				*				**	*
	Compliance with Kyoto				*		*	*		*
ABANDONMENT	Withdrawal of installation		**	**	**	**	**	**	*	*
	Restoration	*	*	*	*	*	*	*	*	*

* = Positive Impacts ** = Negative Impacts

[Tabl.1]

TABLE 2.- CARACTERIZATION IMPACTS OF THE PROJECT



PROJECT PHASES		PHYSICAL ENVIRONMENT					BIOTA MIDDLE		VISUAL IMPACT	ECONOMY AND SOCIETY
		Geomorphology	Surface water	Soil	Atmosphere	Noise pollution	Vegetation	Animals	Visual Impact	Level occupation
CONSTRUCTION	Preparing the ground	CM	C	CM	C	C	CM	CM	C	+
	Civil Works	CM	C	CM	C	C	CM	CM	C	+
	Mechanical work	NS	NS	NS	NS	NS	NS	NS	NS	+
	Electrical workers	NS	NS	NS	NS	NS	NS	NS	NS	+
	Transport of materials and equipment	NS	NS	C	C	C	C	C	C	+
	Land use	C	C	CM	NS	NS	CM	C	C	NS
	Demand for labour	NS	NS	NS	NS	NS	NS	NS	NS	+
	Storage of materials	C	C	C	NS	NS	C	NS	C	NS
	Waste Storage	C	C	C	NS	NS	NS	NS	NS	NS
	Revegetation	+	+	+	+	+	+	+	+	+
OPERATING MODE	Maintenance	NS	NS	NS	NS	NS	NS	NS	NS	+
	Maintenance of electrical equipment	NS	NS	NS	NS	NS	NS	NS	NS	+
	Presence of Solar Park	NS	NS	NS	NS	NS	C	C	C	NS
	Compliance with Kyoto	NS	NS	NS	+	NS	NS	NS	NS	+
ABANDONMENT	Withdrawal of installation	C	C	+	C	C	+	+	+	C
	Restoration	+	+	+	+	+	+	+	+	+

NS Not significant + Positive C Consistent CM Consistent moderate

[Table 2]

TABLE 3.- RATING IMPACT OF THE PROJECT

PROJECT PHASES	ENVIRONMENT	ELEMENT	CARACTERIZATION IMPACTS	VALORATION IMPACTS
CONSTRUCTION	PHYSICAL ENVIRONMENT	GEOMORFOLOGY	CM	Direct Negative, Permanent (long term), Synergistic, Irreversible and Recovery
		SURFACE WATER	C	Direct Negative, Temporary (short term), Synergistic, Reversible and Recovery
		SOIL	CM	Direct Negative, Temporary (long term), Synergistic, Reversible and Recovery
		ATMOSPHERE	C	Direct Negative, Simple, Temporary (short term), Not to continue, and Recovery Reversible
		NOISE POLLUTION	C	Direct Negative, Temporary (short term), not to continue, Synergistic, Reversible and Recovery
	BIOTA MIDDLE	VEGETATION	CM	Direct Negative, Temporary (short and medium term), Synergistic, Reversible and Recovery
		ANIMALS	CM	Direct Negative, Simple, Temporary (medium term), Reversible and Recovery
	VISUAL IMPACT	VISUAL IMPACT	C	Direct Negative, Simple, Temporary (short term), Reversible and Recovery
	ECONOMY AND SOCIETY	LEVEL OCCUPATION	Positive	Direct, Positive, Temporary (short and medium term) and Synergistic
OPERATING MODE	PHYSICAL ENVIRONMENT	SURFACE WATER	NS	
		SOIL	NS	
		ATMOSPHERE	NS	
		NOISE POLLUTION	NS	
		COMPLIANCE WITH KYOTO	+	Direct, Positive, Permanent, Simple and Magnitude High
	BIOTA MIDDLE	VEGETATION	NS	
		ANIMALS	C	Indirect, Negative, Temporary (medium term), Simple, Reversible and Recovery
	VISUAL IMPACT	VISUAL IMPACT	C	Direct Negative, Temporary y (long term), Synergistic, Reversible and Recovery
	ECONOMY AND SOCIETY	LEVEL OCCUPATION	Positive	Direct, Positive, Standing (short and medium term), Simple and Magnitude High
ABANDONMENT	PHYSICAL ENVIRONMENT	GEOMORFOLOGY	C	Direct Negative, Permanent (long term), synergistic, Irreversible and Recovery
		SURFACE WATER	NS	
		SOIL	C	Direct Negative Temporary (short term), Synergistic, Irreversible and Recovery
		ATMOSPHERE	C	Direct Negative, Temporary (short term), Simple, Not continuous, Reversible and Recovery
		NOISE POLLUTION	C	Direct Negative Temporary (short term), Simple, Not continuous, Reversible and Recovery
	BIOTA MIDDLE	VEGETATION	C	Direct, Positive, Temporary (short term), Synergistic, Reversible and Recovery
		ANIMALS	C	Direct Negative Temporary (short term), Synergistic, Reversible and Recovery
	VISUAL IMPACT	VISUAL IMPACT	C	Direct, Positive, Temporary (short term), Simple, Reversible and Recovery
	ECONOMY AND SOCIETY	LEVEL OCCUPATION	C	Direct Negative, Temporary I (medium and long term), Synergistic, Recovery and Reversible

[Table 3]

The project of 1.5 MW photovoltaic installation connected to the grid, in the municipality of Blanca (Murcia, Spain), will produce different impacts on different elements of the environment. The negative effects will be low or very low magnitude, in ways physical, biological and landscape, while in the midst socioeconomic will generate positive effects.

The proposed action is environmentally viable and impacts produced by this installation are acceptable. After assessing the effects, should be put in the necessary corrective and protective measures to avoid and / or minimize the impacts generated by the proceedings and, furthermore, comply with the Plan of Environmental Monitoring.

Finally, Park Photovoltaic Solar entails:

1. Increase the capacity of generation of electricity supply and improve the reliability and quality of the same in the Region of Murcia.
2. Improving the competitiveness of the economy of Blanca, reducing the rate of foreign energy dependence.
3. Supporting the generation of employment in the town of Blanca, improving the quality of life for all citizens.
4. Respect the environment with clean energy and renewable.

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Seasonal biomonitoring of a contaminated coastal Lagoon (Ria de Aveiro, Portugal) assessing 8-hydroxyguanine, DNA strand breakage and erythrocytic nuclear abnormalities in Dicentrarchus labrax L.

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Aims: Ria de Aveiro has been the main receptor of anthropogenic discharges resulting mainly from industries (chlor-alkali and pulp/paper plants), harbours and dry-dock activities as well as municipal effluents. In this perspective, Dicentrarchus labrax L. 8-hydroxy-2'deoxyguanosine (8-OHdG), which is an oxidative free radical induced DNA lesions resulting from the oxidation of guanine in the C8 position, along with DNA strand breakage and erythrocytic nuclear abnormalities (ENA) assays, as measures of genetic damage, were applied to monitor the presence of genotoxic contaminants in this complex water pollution scenario.

Methods: Sampling: European sea bass (Dicentrarchus labrax L.) were caught in the Spring, Summer, Autumn and Winter of 2006, during low tide using fishing rod. Sea bass capture was carried out at six locations (Torreira-TOR, Barra-BAR, Gafanha-GAF, Rio Novo do Príncipe-RIO, Laranjo-LAR, Vagos-VAG) along the lagoon. Among all the study sites, TOR was selected as a reference site due to the absence of known input points and because it is far from the main polluting sources. Juvenile specimens were selected on the basis of their size. Biometrical parameters, such as fish weight and total length ranged from 18.71 - 41.96 g and 12.9 - 16.5 cm, respectively. Blood was collected from the posterior cardinal vein using a heparinized Pasteur pipette and used for smear preparation and submitted to DNA isolation procedure, as well as plasma separation using an Eppendorf centrifuge (14 000 rpm).

8-hydroxy-2´-deoxyguanosine (8-OHdG) measurement: 8-OHdG resulting from oxidative damage to DNA was quantified in plasma through a competitive enzyme-linked immunosorbent assay using an IBL (Hamburg) kit.

DNA alkaline unwinding assay: Deep frozen portions of blood were submitted to DNA isolation procedure (Genomic DNA Purification Kit, Fermentas). DNA integrity measurements were performed according to Rao et al. (1996) as previously adapted by Maria et al. (2002). DNA single and double strands were separated by hydroxyapatite (20%) "batch" elution and quantified with a DNA-specific fluorescent dye (2.5 nM bisbenzimide), after alkaline unwinding. Data from DNA unwinding technique were expressed as an F-value [DNA integrity (%)], determined by applying the following equation: $F = (ds/(ds+ ss)) \times 100$ where ss is the relative fluorescence of the single-stranded eluent of a sample minus the single-stranded control blank fluorescence value, and ds is the relative fluorescence of the corrected double stranded eluent of the same sample.

ENA assay: Blood smears were fixed with methanol during 10 minutes and stained with Giemsa (5%) during 30 minutes. The nuclear abnormalities were scored in 1000 mature erythrocytes sample per fish, according to the criteria of Schmid (1976), Carrasco et al. (1990) and Smith (1990), adapted by Pacheco and Santos (1996). According to these authors, nuclear lesions were scored into one of the following categories: micronuclei, lobed nuclei, dumbbell shaped or segmented nuclei and kidney shaped nuclei. The final result was expressed as the mean value (%) of the sum for all the individual lesions observed.

Integrated biomarker response (IBR): A method for combining all the measured biomarker responses into one general "stress index" termed "Integrated Biomarker Response" (IBR), (Beliaeff and Burgeot, 2002; Broeg and Lehtonen, 2006) was applied for the observation of the extent of pollution in each season. Statistical analysis: Results are expressed as means ± SE (standard error) corresponding to experimental groups of six fish (n=6). The DNA integrity data were transformed prior to statistic analyses according to the formula arcsen $\sqrt{p}$ The experimental data were tested first for normality and homogeneity of variance to meet statistical demands and then with the Tukey test (Zar, 1999). Differences between means were considered significant at $P<0.05$.

Results: Seasonal plasma 8-OHdG content assessment in D. labrax displayed a significant increase in its content in Spring at GAF, RIO, LAR and VAG when compare to TOR. In Summer, no significant difference in plasma 8-OHdG content was observed at all the sites when compared to TOR. In Autumn, a significant induction was observed only at GAF whereas in Winter season, a significant

plasma 8-OHdG content decrease was recorded at BAR and GAF compared to TOR.
Concerning DNA damage, a significant DNA integrity decrease was observed only in spring season at GAF and VAG comparing to reference sites.
In the perspective of ENA, a significant induction was observed at BAR, LAR and VAG in spring season whereas fish collected in summer showed a pronounced ENA induction at all the study sites except GAF when compared to TOR. In autumn, ENA induction was significant at LAR and VAG whereas in winter, GAF and RIO were the sites showing a significant ENA induction.
Overall the joint data integration using IBR exhibited the following ordering of surveyed sites according to the contamination degree and risk to fish health: Spring - LAR > VAG > RIO > BAR > GAF > TOR; Summer - GAF > VAG > LAR > RIO > TOR; Autumn - VAG > LAR > RIO > TOR > GAF; Winter - GAF > RIO > TOR > BAR.
Conclusions: Aveiro lagoon seasonal assessment using combined approach of 8-OHdG, DNA strand break and ENA reflected the presence of anthropogenic contaminants having genotoxicity inducing potential and demonstrated that there is a serious risk to fish populations probably due to mixtures of xenobiotics and the type of interaction (synergism/potentiation or antagonism) in a particular mixture varying the evaluated biological response.
Oxidative stress intervention as a cause of genotoxicity induced by water contaminants was confirmed since 8-OHdG induction, which is a typical modification of DNA caused by oxygen free radicals, was significantly increased in sites GAF, RIO, LAR and VAG (Spring); GAF (Autumn); as well as BAR and GAF (Winter). Thus, all the surveyed sites were ordered according to the contamination degree and risk to fish health.
The current findings reflect the ability of D. labrax for biomonitoring aquatic anthropogenic contaminants on the basis of the integrated approach of 8-OHdG, DNA strand break and ENA assessment and can be successfully employed in both Atlantic and Mediterranean European coastal waters. D. labrax revealed to be a particularly appropriate species for genotoxicity monitoring of heterogeneous genotoxic contamination in coastal ecosystems.

P 25

Respiratory toxicity of raw materials used in the manufacture of sanitary wares

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Objective: The main purpose of this study was to assess the pulmonary reactions associated with exposure to raw materials used in the production of sanitary wares (RMPS).
Methodology: This was a cross sectional study in which 33 male workers with current exposure to RMPS and 20 healthy male unexposed workers (referent group) were interviewed and respiratory symptom questionnaires were administered to them. Furthermore, they underwent chest X-ray and lung function tests. Additionally, personal dust monitoring for airborne inhalable and respirable dust was carried out at 12 different work sites. To determine the chemical composition, possible silica phases and SiO2 contents of dust samples, they were analyzed by X-ray diffraction (XRD) and X-ray fluorescence (XRF) techniques.
Results: The average (Mean ± SD) age (years), weight (kg), height (cm) and duration of exposure to dust (years) for the exposed group were 31.3 ± 4.2, 68.8 ± 10.2, 175.7 ± 7.6 and 6.5 ± 2, respectively. The corresponding values for the referent group were 41.7 ± 5.8, 76.4 ± 11.6, 172.5 ± 7.8 and 0 ± 0, respectively. Chemical analysis of the samples revealed that their crystalline silica phase was Quartz and they contained 69% SiO2. Similarly, levels of exposures to inhalable and respirable dust were estimated to be 71.7 ± 16.2 and 26.7 ± 19.2 mg/m^3, respectively. Respiratory symptom questionnaires revealed that exposed workers, compared to their unexposed counterparts, had higher prevalence´s of cough, wheezing, phlegm and shortness of breath. Similarly, their chest radiographs showed various degrees of abnormalities. However, no significant changes were noted in the radiographs of unexposed employees. Likewise, significant reductions in some parameters of pulmonary functions of exposed workers were noted.

Conclusions: These data provide further evidence in favor of the notion that exposure to RMPS, probably due to their silica contents, is associated with respiratory symptoms, radiographic abnormalities and functional impairments. Therefore, using engineering methods and /or personal protective respirators occupational and environmental exposure to this toxic dust should be prevented or minimized.

P 26

Respiratory disorders associated with heavy inhalation exposure to dolomite dust

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Dolomite, calcium magnesium carbonate with the chemical formula CaMg(CO3)2, has many applications in construction, building product, asphalt and concrete as well as an agent for agricultural pH control.
Although this compound is classified as a relatively non-toxic, nuisance dust, little information exists as to its potential health risks following heavy exposure. This study was, therefore, undertaken to address this issue. The study population consisted of a group of 39 exposed subjects engaged in digging and excavating activities that were in operation for building a local dam, as well as 40 healthy unexposed employees that served as the referent group. Subjects were interviewed and respiratory symptom questionnaires, as suggested by the American Thoracic Society (ATS), were completed for them. Thereafter, they underwent chest X-ray and lung function tests. Additionally, using standard methods, personal dust monitoring for airborne inhalable and respirable dust was carried out at different dusty work sites. Finally, to determine the chemical composition of the dust, it was analyzed by X-ray diffraction (XRD) and X-ray fluorescence (XRF) techniques.
Chemical analysis of the dust revealed that its major component (50.52%) was calcium magnesium carbonate. Additionally, levels of exposures to inhalable and respirable dust were estimated to be 51.7 ± 24.3 and 23 ± 18.1mg/m^3, respectively (Mean ± SD).
Statistical analysis of the data showed that symptoms like regular cough, phlegm , wheezing , productive cough and shortness of breath were significantly ($P< 0.05$) more prevalent among espoused workers. Similarly, a significant reduction in the ratio of FEV1 to FVC of exposed subjects as compared to their non exposed counterparts was noted. In contrast, no significant abnormalities were observed in the chest radiographs of both groups.
In conclusions, while these data cast doubt on the notion that dolomite is a harmless chemical, provide evidence in favor of the proposition that exposure to high atmospheric concentrations of this compound is likely to be associated with respiratory symptoms and functional impairments. Therefore, using engineering methods and/or personal protective equipment occupational exposure to this chemical should be prevented or minimized.
Acknowledgement: The authors wish to sincerely thank Dr. MA Ghayomi, pneumologist, for reading and reporting the radiographs and spirograms.

P 27

Liza aurata intermediary metabolism and hormonal responses in Ria de Aveiro pollution monitoring - a seasonal study

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Aims: Alterations in thyroid hormones and cortisol plasma concentrations, as well as on glucose and lactate levels can reflect endocrine alterations induced by pollutants, reducing fish physiological competence and possibly survivorship. The objective of this study was to assess the thyroid-stimulating-hormone (TSH), the thyroid hormones (triiodothyronine - T3 and free thyroxine T4) and stress (cortisol, glucose and lactate) responses, in Liza aurata caught at Ria de Aveiro, over the period of one year.

Methods: Study Area: The Ria de Aveiro is a shallow estuary that is permanently connected to the ocean. The sampling sites were selected on a geographic distribution basis along the main channels taking into account the various types and sources of contamination as well as the selection of a (theoretically) unpolluted reference point. The sampling sites were: Torreira (TOR), an intermediate region of the longest channel (S. Jacinto-Ovar channel), far from the main polluting sources and thus assumed as the reference site; Barra (BAR), the initial part of the Mira channel close to the lagoon entrance and subject to a considerable naval traffic; Gafanha (GAF) situated in the vicinity of a deep-sea fishing port and dry-docks, also connected with the main channel coming from Aveiro city carrying domestic discharges; Rio Novo do Principe (RIO), located at the terminal area of the Vouga River, 6.5 km distant from a pulp/paper mill effluent outlet, that discharged to this water course during nearly five decades (until the year 2000); Laranjo (LAR), close to a chlor-alkali plant (6 km), an important source of metal contamination (mainly $HgCl_2$); Vagos (VAG), located at the terminal part of the Ílhavo channel, receiving municipal and domestic effluents with high levels of PAHs. Sampling: Golden grey mullet (Liza aurata) was caught in the Spring, Summer, Autumn and Winter of 2006, during low tide using a traditional beach-seine net named “chincha”. Juvenile specimens were selected on the basis of their size. Biometrical parameters, such as fish weight and total length ranged from 18.71 - 41.96 g and 12.9 - 16.5 cm, respectively. Blood was collected from the posterior cardinal vein using a heparinized Pasteur pipette and used for smear preparation and submitted to DNA isolation procedure, as well as plasma separation using an Eppendorf centrifuge (14 000 rpm).

Hormonal responses measurement: The determinations of cortisol, TSH, T3 and T4 were performed in plasma, using diagnostic ELISA direct immunoenzymatic kits (Diametra, Italy). The absorbance was measured at 450 nm in a microplate reader (ASYS Hitech). The cortisol in the sample competes with horseradish peroxidase (HRP)-cortisol for binding onto the limited number of anti-cortisol sites in the microplate wells. The enzyme substrate (H_2O_2) and the TMB (3,3´, 5,5´ tetramethylbenzidine)-substrate are added, and after an appropriate time has elapsed for maximum color development, the enzyme reaction is stopped and the absorbances are determined. Cortisol concentration in the sample is calculated based on a series of standards and the color intensity is inversely proportional to the cortisol concentration in the sample. The methods for free T3 and free T4 follow the same principles of the cortisol test, requiring immobilized T3 or T4 antibodies, as well as HRP-T3 or HRP-T4 conjugates. Concerning TSH, an antibody specific to the h-chain of TSH molecule is immobilized on microwell plates and other antibodies to the TSH molecule are conjugated with HRP. TSH from the sample is bound to the plates. The enzymatic reaction is proportional to the amount of TSH in the sample.

Intermediary metabolism responses measurement: Plasma glucose was measured spectrophotometrically (340 nm) according to the method modified from Banauch et al. (1975), based on the quantification of NADH after a glucose oxidation catalysed by the glucose-dehydrogenase. The quantity of NADH formed is proportional to the glucose concentration. Plasma lactate levels were determined spectrophotometrically (340 nm) according to the method modified from Noll (1974) using LDH, ALT and NAD, measuring the NADH appearance.

Integrated biomarker response (IBR): A method for combining all the measured biomarker responses into one general ‘‘stress index’’ termed ‘‘Integrated Biomarker Response’’ (IBR), (Beliaeff and Burgeot, 2002; Broeg and Lehtonen, 2006). Statistical analysis: Results are expressed as means ± SE (standard error) corresponding to experimental groups of six fish (n=6). A statistical data analysis was done using Statistica software (StatSoft, Inc., Tulsa, OK). The assumptions of normality and homogeneity of data were verified. A factorial analysis of variance (Two-Way ANOVA) was performed in order to assess significant effects in each site and the differences between seasons; this analysis was followed by post-hoc Fisher LSD test to signal significant differences between groups (Zar, 1999). The significance of the results was ascertained at $\alpha = 0.05$.

Results: In Spring, TSH levels were significantly lower than control site (TOR) in BAR and LAR. However, in Autumn TSH levels were found higher than TOR at BAR. T3 levels in Spring were significantly lower than TOR at all studied sites. However, in Summer the fish caught at GAF and VAG displayed significantly higher T3 levels though at RIO they remained lower than TOR. In Autumn, BAR and LAR levels were significantly lower than TOR whereas in Winter RIO, LAR and VAG displayed significantly lower levels than TOR.

T4 levels at BAR and RIO were significantly higher than at TOR whereas at GAF, LAR and VAG T4 was significantly lower. In Summer and Winter, all sites displayed significantly lower T4 levels than TOR though no significant changes were detected in Autumn.

Cortisol levels were significantly decreased, when compared to the reference site, at BAR, GAF, LAR, VAG in Spring; in all sites in Summer; and in GAF and RIO in Autumn. However, in Winter at GAF, RIO, LAR and VAG, cortisol levels were significantly higher than TOR whereas at BAR, cortisol level was significantly decreased.

Concerning intermediary metabolism responses, glucose levels were significantly higher than TOR at BAR, GAF and VAG in Spring, at GAF in Summer and Winter. However, in Winter, a significantly lower glucose level was found at BAR.

Concerning lactate, significantly higher levels were found at LAR and VAG in Spring and BAR, RIO, LAR and VAG in Summer. In Autumn and Winter no significant differences to control site were found. No significant differences between seasons were found in the control site in the intermediary metabolism responses though observed in the contaminated sites. However, in terms of hormone responses, seasonal changes were observed in all the studied sites.

The IBR index values in Spring and Summer were found higher respectively in VAG and GAF. In Autumn and Winter, IBR values were higher at BAR.

Conclusions: The studied biomarkers identify critical points in the Aveiro lagoon. A wide range of mechanisms can be implicated in the xenobiotic-induced alterations of plasma thyroid hormones, corresponding to changes on thyroid status and/or alterations upstream or downstream the hormone production. Overall, the responses confirm that the effects of the waterborne chemical compounds are influenced by abiotics factors that may change their bioavailabilty. L. aurata demonstrated the capacity to respond to the environmental changes. Considering the above findings, it can be suggested that fish populations living in the current studied areas can suffer impairment of several biological functions.

P 28

Evaluation of respiratory effects of exposure to synthetic vitreous fibers (SVF)

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Despite the wide environmental applications of synthetic vitreous fibers (SVF), the potentials of this compound to induce respiratory syndromes, ventilatory impairments or abnormal radiographic changes in the lungs has not been extensively studied and is subject to debate and controversy. This study was, therefore, undertaken to address this issue. The study population consisted of a group of 49, randomly selected, male workers with current occupational exposure to SVF as well as 45 healthy male office workers without history of past or present exposure to this compound (referent group). The average (Mean ±SD) age (years) and the duration of exposure to dust for the exposed group were 39.6±7.3 and 11.4±5.6, respectively. The corresponding values for the control group were 42.8±7.6 and 0±0, respectively. Subjects were interviewed and respiratory symptom questionnaires, as suggested by the American Thoracic Society (ATS, 1978), were completed for all of them. They were classified to smokers and non-smokers and underwent chest X-ray and lung function tests according to the guidelines given by the ATS, 1979. Furthermore, using standard methods, personal dust monitoring for airborne inhalable and respirable fractions was carried out at different worksites. Atmospheric concentrations of SVF in the breathing zone of exposed workers were estimated to be 44.5 and 6.27 mg/m^3 in line and tissue units, respectively. Analysis of the data revealed that there was no significant difference between the prevalence of respiratory symptoms among exposed and unexposed workers. Similarly, the prevalence of abnormal radiographic findings such as chronic inflammatory process, calcification and fibrosis in both groups was not significantly different. Furthermore, the results of spirometry demonstrated that lung function parameters in exposed workers were not significantly different from those of unexposed population. These data indicate that under the above-mentioned exposure scenario SVF is unlikely to induce respiratory symptoms or ventilatory disorders.

P 29

Treatment and reuse of urban sewage in phosphate industry

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Demographic changes and urban development combined with the rural exodus movement and drought confront Morocco to a risk of water shortage by 2025. Currently the ratio of water resources in Morocco east of 950 m^3/habitant/an, below this threshold scarcity of water resources set by the United Nations in 1000 m^3/habitant/an. In Morocco the phosphate industry uses large quantities of water and does not stop to increase with the development of the sector.
The region of Khouribga (Morocco), or is the exploitation of phosphate, has a deficit of water and hence the need to mobilize resources from non-conventional water, namely the reuse of treated urban wastewater for washing and flotation of phosphates. In order to respond to a water quality objective, we have undertaken several scenarios treatment. The best results were achieved by a combination of natural lagoons with infiltration percolation and combination of activated sludge with infiltration percolation.

P 30

Contribution to the solubilization of phosphorus via micro-organisms of the ground

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For the plants, the phosphorus is an essential system for energy transfer. This mineral is abundant in soil but because of its intense reactivity, the phosphorus available amount for plants is a growth limiting factor. Phosphorus is sequestered through precipitation and adsorption phenomena. It is able to be adsorbed on soil particle surface and reacts with cations, especially iron, aluminium and calcium as well as with insoluble compounds, hindering its plant use.
Microorganisms are able to solubilise phosphorus and may biotransform the insoluble phosphate into soluble form through acidification, chelating and exchange reactions. In this study, bacterial strains able to solubilise phosphorus were isolated from natural environment.
The characterization of these strains was made by screening tests based on solid media including phosphates (NBRIP) and bromophenol blue. The culture of the efficient strains in liquid media was performed and showed that the liquid medium exhibited a better bacterial growth and solubilisation of the insoluble phosphate. This activity decreased the medium pH and was demonstrated to be dependent on the initial phosphorus concentration used. This solubilisating activity was related to peptides evidenced in the cultural media. These were partially purified.

P 31

Development of methods for determination of Cd, Cu, Ni and Pb in sea water by FAAS

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Methods have been developed for determination of cadmium, copper, nickel and lead in sea water by flame atomic absorption spectrometry using on-line preconcentration with immobilized 8-quinolinol. The effect of pH on Cd, Cu, Ni and Pb up take by the column was investigated after buffering the solution of metal ions with 0.1 mol/l acetate. The optimum pH values for preconcentration of Cd, Cu, Ni

and Pband using 8-quinolinol resin were 8, 7, 8 and 6 respectively.
The effects of salt ions as Na, k, Ca, Mg, Cl and SO4 at different concentrations on Cd, Cu, Ni and Pb up take by the column were also investigated. The percentage recovery obtained in presence of 100 mg/l potassium was between 70% for Cu and 98% for Cd, in presence of 500 mg/l sodium was between 78% Cu and 99% for Cd, in presence of 100 mg/l calcium was between 65% for Ni and 99% for Cd, in presence of 100 mg/l magnesium was between 58% for Ni and 96% for Cd, while in presence of 1000 mg/l chlorine was between 80% for Cu and 99% for Cd and in presence of 500 mg/l sulfate was between 63% for Cu and 100% for Cd.
Finally the methods developed for determination of Cd, Cu, Ni and Pb were applied for analysis of sea water samples collected from different sites along Tripoli coast. The lowest concentration levels observed for Cd, Cu, Ni and Pb were 2.3, 6.25, 22.2 and 4.16 µg/l respectively, while the highest concentration levels were 74.3, 16.67, 45.14 and 64.24 µg/l respectively.

P 32

A factor analysis study: Air pollution, meteoroloy and hospital admissions for respiratory diseases

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It is know that air pollution has adverse effects on the health and environment. Balıkesir city located in the Marmara region (Turkey) has serious air pollution problems which caused by heating in winter months. In this study, It has been investigated the effect of exposure to air pollution on hospital admission for respiratory illnesses among children and adults. Epidemiological data from records of hospitals, air pollutants and meteorological data were used. During July 2005-July 2005 period, there was a total of 280.426 hospital admissions for respiratory diseases. In a population-based analysis, 9 children were admitted for asthma, 17 children for acute bronchitis, 20 children for lower respiratory diseases per 100 children. There was a significant increase in the hospital admissions for respiratory diseases and air pollutants from October to March. It has been concluded that children living in city suffer from respiratory diseases such as asthma, pneumonia and lower respiratory diseases. It has been identified 3 factors that explain approximately 90% of total variance of hospital admissions, air pollution and meteorology by means of principal component factor analysis.

P 33

Respiratory toxicity of kiln precipitator catch

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The potential adverse pulmonary effects of kiln precipitator catch (portland cement) have not been thoroughly investigated. This study was, therefore, undertaken to ascertain if there is any association between exposure to this compound and respiratory symptoms and/ or ventilatory disorders. Two groups of workers, case and control, were randomly selected from employees of a local plant. They were interviewed, respiratory symptom questionnaires were administered to them, and underwent chest X-ray and pulmonary function tests. Additionally, using standard methods, airborne concentrations of dust were measured at several points. Moreover, the silica phases and the SiO2 contents of dust samples were determined by standard techniques. Analysis of dust samples showed that they contained more than 1% of SiO2. Level of exposure to dust was far beyond the current standards. Statistical analysis of the data showed that symptoms such as regular cough, phlegm, wheezing and shortness of breath and abnormal radiographic findings such as emphysematous and infiltrative changes were significantly more common in exposed group than in control subjects. Similarly, the results of spirometry demonstrated statistically significant reduction in lung function parameters (i.e; VC, FVC, FEV1, PEF) in exposed subjects as compared to controls. Overall, the data provide evidence in favour of the notion that exposure to this chemical is likely to be associated with respiratory symptoms, radiographic changes and functional impairments.

P 34

Determination of polycylic aromatic hydrocarbons in chicken meat, liver and eggs

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Introduction: Chicken meat and eggs are low cost protein sources that are widely used in human nutrition. The contamination of chicken meat or eggs with organic pollutants in industrial aviaries is normally prevented because of the highly standardized production conditions. The same is not true for the so-called wide range chicken that grow in outdoor spaces and are exposed to environmental conditions namely the degree of contamination of air, soil or water.
G. Schoeters and R. Hoogenboom (1) published a review of the studies on the contamination of free-range chicken eggs with dioxins and dioxin-like polychlorinated biphenyls. Significant contamination was found in eggs from free-range chicken, and in particular organically and home-raised ones. Shore et al. (2) reported the presence of PAHs in coastal-nesting birds from Britain. The contamination of chicken and their eggs with dioxins or PCBs is also reported by Hayward et al. (3), Traag et al. (4), Zhao et al. (5) and Stephens et al. (6), among others. The purpose of this work is to optimize the conditions for the extraction and analysis of 16 PAHs in chicken meat, liver and eggs from home-raised chicken in the area of Sines, Portugal. Significant PAH contamination of the air and soil occurs in this area because of the concentration of industrial equipments namely oil refineries. The extraction and clean-up procedure involved saponification, liquid-liquid extraction and solid phase extraction. Chromatographic analysis was performed using gas chromatography and mass spectrometry. The limits of detection and quantification of the chromatographic analysis were determined. The solid phase conditions were optimized for the standards and for each one of the matrices studied and the recoveries of the clean-up step were determined. The recoveries of the overall process were also determined for the three types of samples.
Methods: Chicken liver, meat and eggs were purchased from a Lisbon local market and from Sines particular producers.
The samples (approximately 20 g for eggs and 80 g for meat and liver) were subjected to transesterification with methanolic KOH, followed by liquid-liquid extraction with cyclohexane and partition with dimethylformamide. The extracts were dried with anhydrous sodium sulphate and concentrated to 1mL before SPE clean-up. A volume of 1 mL of the standard solution of the PAHs or of samples spiked at 1ppm level were loaded into the SPE column (2,5g) and the PAHs were eluted using: n-hexane, mixtures of n-hexane and acetone or mixtures of n-hexane and dichloromethane (DCM). All extracts were concentrated to 1 mL before GC-MS analysis and spiked with 0.1 mL of perylene-d12 standard solution (internal standard). Chicken meat, liver and eggs were spiked with a mixture of the 16 EPA PAHs at a concentration level of 10 µg/L and subject to the overall process of extraction and analysis. The SPE elution was performed with 15 mL of n-hexane:dichloromethane (9:1). Non spiked samples were also analyzed with the same methodology. All samples were concentrated to 1mL, spiked with perylene-d12 and analysed by GC-MS. The chromatographic analysis were performed using a Focus gas chromatograph equipped with a split-splitless injector, a DB5-MS capillary column (30 m × 0.25 mm I.D. × 1.4 µm film) and a Polaris Q mass spectrometer detector.
Results: The instrumental limits of detection (LODs) and limits of quantification (LOQs) were determined for the 16 PAHs (Table 1). The LOQs were in the range of 4.4 to 28.4 µg/L with the exception of the dibenzo(a,h)anthracene with 114.9 µg/L . With a ten-fold concentration of the PAHs present in the samples overall LOQs for the process will be below 5 µg/L which is the maximum admissible concentration for many food matrices, in particularly meat and eggs.

Analyte	LOD (μg/L)	LOQ (μg/L)	Analyte	LOD (μg/L)	LOQ (μg/L)
Naphthalene	4.1	12.5	Benzo[a]anthracene	3.6	10.8
Acenaphthylene	4.3	12.9	Chrysene	6.1	18.5
Acenaphthene	5.1	15.4	Benzo[b]fluoranthene	5.6	17.0
Fluorene	6.7	20.2	Benzo[k]fluoranthene	6.7	20.2
Phenanthrene	3.4	10.4	Benzo[a]pyrene	8.2	24.8
Anthracene	4.3	13.0	Benzo[ghi]perylene	9.4	28.4
Fluoranthene	1.5	4.4	Indeno[1,2,3-cd]pyrene	7.4	22.4
Pyrene	2.5	7.7	Dibenzo[a,h]anthracene	37.9	114.9

Navarro et al. (7) compared the performance of different methods of PAHs extraction and clean-up for oysters, mussels and fish liver - these authors suggest the use of multiple clean-up procedures and depict the influence of matrices effects.

For the chicken products studied in this work the solid phase extraction step was optimized by varying the nature and amount of the solid adsorbent and the nature and volume of the eluent. For 80g of meat or liver samples and for 30g of egg samples, at least 2.5g of adsorbent (silica or florisil) were necessary to efficiently remove the fatty acid methyl esters. Silica produced extracts with less interferences than the florisil cleaned extracts and with comparable analyte recoveries, so it was chosen as adsorbent for the remaining tests.

Elution of the PAHs was performed using hexane, hexane:acetone (9:1 and 8:2) and hexane:DCM (9:1 and 8:2). The 9:1 hexane:DCM mixture was chosen as eluent because it provided good recoveries and cleaner chromatograms when compared with the other eluents (Table 2).

Analyte	Standard solution	Chicken meat	Chicken liver	Chicken eggs	Analyte	Standard solution	Chicken meat	Chicken liver	Chicken eggs
Naphthalene	94.8	109.9	137.1	84.2	Benzo[a]anthracene	101.8	75.3	114.6	83.2
Acenaphthylene	105.6	81.8	108.1	80.7	Chrysene	106.1	75.2	101.8	81.4
Acenaphthene	104.8	80.2	108.2	80.5	Benzo[b]fluoranthene	96.4	46.8	94.7	72.6
Fluorene	109.1	81.8	114.8	79.7	Benzo[k]fluoranthene	97.2	55.6	88.1	75.1
Phenanthrene	109.1	92.6	113.0	87.7	Benzo[a]pyrene	102.0	72.1	94.0	88.2
Anthracene	111.9	96.5	111.4	90.9	Benzo[ghi]perylene	96.1	38.5	90.9	63.6
Fluoranthene	108.0	87.7	111.0	85.3	Indeno[1,2,3-cd]pyrene	101.7	50.3	80.3	67.1
Pyrene	103.4	90.8	111.2	86.2	Dibenzo[a,h]anthracene	92.3	64.5	101.2	38.2

Finally the overall process was applied to chicken meat, liver and egg samples spiked with known amounts of the 16 PAHs. The recoveries evaluated after correction of the PAHs levels in non spiked samples were extremely variable in what concerns the nature of the sample and the individual PAHs (Table 3).

Analyte	Chicken meat	Chicken liver	Chiken eggs	Analyte	Chicken meat	Chicken liver	Chicken eggs
Naphthalene	165.1	30.2	75.6	Benzo[a]anthracene	45.5	31.3	<1
Acenaphthylene	128.8	52.5	<1	Chrysene	45.6	38.0	<1
Acenaphthene	159.7	65.6	<1	Benzo[b]fluoranthene	8.0	6.1	<1
Fluorene	150.8	71.6	<1	Benzo[k]fluoranthene	14.2	15.1	<1
Phenanthrene	160.7	71.1	3.5	Benzo[a]pyrene	16.3	13.9	<1
Anthracene	164.7	73.2	<1	Benzo[ghi]perylene	<1	<1	<1
Fluoranthene	100.5	52.0	1.4	Indeno[1,2,3-cd]pyrene	<1	<1	<1
Pyrene	113.6	54.1	1.3	Dibenzo[a,h]anthracene	<1	<1	<1

With this method recoveries decreased with PAH molecular weight, with values below 50% for the heavier ones. Recoveries of the lighter PAHs for the chicken meat samples were considerably above 100% denoting insufficient clean-up.

The chicken liver samples, on the other hand, showed recoveries between 30.2 and 73.2% for PAHs lighter than chrysene, which are reasonable values for this kind of analytes. Finally the recoveries of the chicken egg samples were less than 1% to 3.5% for all analytes, except naphthalene. This indicates that matrix associated effects affected liquid-liquid extraction and partition steps with the loss almost complete of the PAHs spiked in these samples.

In conclusion the SPE clean-up conditions and chromatographic conditions optimized in this work are adequate to the determination of the 16 PAHs tested at levels below 5 µg/Kg in chicken meat, liver and egg samples.

The extraction and partition methods although frequently described in the literature did not give uniform recovery results for the different samples and different analytes.

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P 35

The impact of oak and beech leaf extracts on freshwater charophyte cell Nitellopsis obtusa

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Biodegraded and/or decomposed leaf litter has great influence on biochemical cycling. During the leaf decay, many chemical compounds are released into the water, which may influence an aquatic ecosystem and in particular water flora. A part of these compounds are quinoid structures capable of generating de novo and/or increasing production of reactive oxygen species, and disturbing the cell internal oxidative balance. It has been found that oak leaf extract when applied for 24-h at dissolved organic carbon (DOC) concentrations from 0.245 to 122.5 mg/L induced activity alterations of oxidative stress enzymes, such as superoxide dismutase, catalase, cytosolic and microsomal glutathione S-transferase and glutatione reductase in freshwater charophyte Nitellopsis obtusa (Grigutytė et al., 2008, accepted). The data are lacking on what extent (if any) and what concentrations the leaf extracts could influence other functions of the algal cell. We investigated electrophysiological and lethality effects of collected in Berlin (near Lake Müggelsee) leaf extracts of oak Quercus robur (7.5-1838 mg/L DOC) and beech Fagus sylvatica (33-1100 mg/L DOC) on the freshwater charophyte algae Nitellopsis obtusa (Desv.) J. Groves.

Bioelectrical activity of living cells of N. obtusa collected from Lake Švenčius (nearby Vilnius) was measured according to the K+-anaesthesia method modified for multichannel recording with extracellular chlorinated silver wire electrodes. The measuring set-up includes two separate Plexiglass chambers for recordig the bioelectrical signals from 32 cells each, analogue signal blocks and controller block connected to PC, for the visualization and analysis of the data. The observation of electrophysiological response was conducted within up to 24-h exposure to a given concentration of the leaf extracts and up to 3-h rewash in control solution.

Lethality response of algal cells were investigated during 30-day exposure. Single internodal cells (each 4-10 cm in length) were placed in Petri dishes (10 cells per dish, 4 replicates), preadapted for 1-

2 days in artificial pond water (APW) containing 0.1 mM KH_2PO_4, 1.0 mM $NaHCO_3$, 0.4 mM $CaCl_2$, 0.1 mM $Mg(NO_3)_2$ and 0.1 mM $MgSO_4$ (pH 7-7.4, unbuffered), and then were kept at room temperature (18-24°C) in the dark. In all treatment s, survival of the cells was checked by gently picking up each cell with a spatula. A cell was judged to be dead when picked up if there was disappearance of turgor pressure, a state in which a cell bends on the spatula and looses its cylindrical shape.

The oak leaf extract induced rapid and significant depolarization of cell resting potential (RP) after exposure to 245 - 1838 mg/L DOC, and this depolarization was permanent during 24-h treatment. The beech leaf extract showed similar fast depolarization of RP after 15-min exposure to 77-1100 mg/L DOC, however this depolarization was transient up to DOC concentrations of 330 mg/L. After 24-h exposure to leaf extracts, a better recovery of cells RP from oak leaf extracts than of beech was observed within a 3-h rewash period.

The cells survived for 1 and 2 days in all tested concentrations of beech and oak leaf extracts, respectively. In general, beech leaf extract was more toxic than oak's: at 245 mg/L DOC, 50 %-mortality was reached after 3-day exposure in beech leaf extract, while the same concentration of oak leaf extract was found to be non-toxic. The results of electrophysiological and lethal responses to leaf extracts will be discussed in relation to previous data obtained on oxidative stress enzymes of charophyte cell.

P 36

Dicentrarchus labrax L. plasma cortisol, thyroid-stimulating-hormone, free thyroxine, free triiodothyronine, glucose and lactate responses to contaminated coastal waters

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Aims: The aim of the present study was the biomonitoring of a polluted coastal lagoon, Ria de Aveiro (Portugal), by assessing hormonal and intermediary metabolic responses in European sea bass (Dicentrarchus labrax L.).

Methods: European sea bass (Dicentrarchus labrax L.) were caught in autumn of 2005 at five location (Torreira-TOR, Barra-BAR, Gafanha-GAF, Rio Novo do Príncipe-RIO, Laranjo-LAR, Vagos-VAG) along the lagoon, using fishing rod. Among all the study sites, TOR was selected as a reference site due to the absence of known input points and because it is faraway from the main polluting sources. Juvenile specimens were selected on the basis of their size. Biometrical parameters, such as fish weight and total length ranged from 18.71 - 41.96 g and 12.9 - 16.5 cm, respectively. Blood was collected from the posterior cardinal vein using a heparinized Pasteur pipette and plasma separation was done using an Eppendorf centrifuge (14 000 rpm). Hormonal responses measurement: The determinations of cortisol, TSH, T3 and T4 were performed in plasma, using diagnostic ELISA direct immunoenzymatic kits (Diametra, Italy). The absorbance was measured at 450 nm in a microplate reader (ASYS Hitech). The cortisol in the sample competes with horseradish peroxidase (HRP)-cortisol for binding onto the limited number of anti-cortisol sites in the microplate wells. The enzyme substrate (H_2O_2) and the TMB (3,3´, 5,5´ tetramethylbenzidine)-substrate are added, and after an appropriate time has elapsed for maximum color development, the enzyme reaction is stopped and the absorbances are determined. Cortisol concentration in the sample is calculated based on a series of standards and the color intensity is inversely proportional to the cortisol concentration in the sample. The methods for free T3 and free T4 follow the same principles of the cortisol test, requiring immobilized T3 or T4 antibodies, as well as HRP-T3 or HRP-T4 conjugates. Concerning TSH, an antibody specific to the h-chain of TSH molecule is immobilized on microwell plates and other antibodies to the TSH molecule are conjugated with HRP. TSH from the sample is bound to the plates. The enzymatic reaction is proportional to the amount of TSH in the sample. Intermediary metabolism responses measurement: Plasma glucose was measured spectrophotometrically (340 nm) according to the method modified from Banauch et al. (1975), based on the quantification of NADH after a glucose oxidation catalysed by the glucose-dehydrogenase. The quantity of NADH formed is proportional to the glucose concentration. Plasma lactate levels were determined spectrophotometrically (340 nm) according to the method modified from Noll (1974) using LDH, ALT and NAD, measuring the NADH appearance.

Integrated biomarker response (IBR): A method for combining all the measured biomarker responses into one general ‘‘stress index’’ termed ‘‘Integrated Biomarker Response’’ (IBR), (Beliaeff and Burgeot, 2002; Broeg and Lehtonen, 2006) was applied for the observation of the extent of pollution in each season. Statistical analysis: Results are expressed as means ± SE (standard error) corresponding to experimental groups of six fish (n=6). The experimental data were tested first for normality and homogeneity of variance to meet statistical demands and then with the Tukey test (Zar, 1999). Differences between means were considered significant at $P<0.05$.

Results: A constant plasma cortisol level was observed at all the lagoon sites, when compared to TOR. Plasma TSH was significantly decreased at LAR. Plasma T3 was significantly increased at GAF and RIO sites when compared to the reference site. Conversely, plasma T4 was decreased significantly at GAF and RIO. Concerning intermediary metabolism responses, a significant plasma glucose decrease was observed only at GAF, whereas plasma lactate was significantly decreased at LAR in comparison to the TOR site. Overall the joint data integration using IBR exhibited that the most impacted sites according to the contamination degree and risk to fish health are LAR and GAF.

Conclusion: Aveiro lagoon water assessment using plasma cortisol, TSH, T3, T4, glucose and lactate revealed indications that lagoon water was contaminated, as evidenced at different study sites: GAF - plasma T3 increase; and T4 as well as glucose decrease; RIO - T3 increase and T4 decrease; LAR - TSH and lactate decrease. Thus, results reflected contamination by a mixture of pollutants from point and non point sources affecting on plasma hormones and intermediary metabolic responses. Fish collected from VAG showed no alteration in all the studied parameters which may be explained due to its ability to cope with the pollutants since this area has domestic influences as well as agricultural activities. The current findings reflect the ability of D. labrax for biomonitoring aquatic anthropogenic contaminants on the basis of hormonal and intermediary metabolism responses and can be successfully employed in both Atlantic and Mediterranean European coastal waters.

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Biomonitoring of mercury exposed subjects in dental clinics

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The findings of some earlier studies, in which high background levels of mercury in the air of dental clinics as well as elevated levels of this compound in the urine and hair of dentists associated with polyneuropathies have been reported, raised concern about dentists in Shiraz over their exposure to amalgam, a restorative agent in dentistry, and prompted this investigation. The study population consisted of 106 dentists and 94 general practitioners (referent group), randomly selected from private and public clinics in Shiraz. Subjects were requested to complete two questionnaires, one on symptoms experienced and amalgam and waste handling and the other on general health. Additionally, using standard methods, atmospheric and urinary concentrations of mercury were measured. The average (mean ± SD) of age (years), weight (kg), height (cm) and length of occupational exposure to amalgam (years) for dentists were 38±8, 68.8±11.1, 168.8±8.2 and 11.7±7.3, respectively. The corresponding values for the referent group were 40.8±7.7, 70.2±10.1, 169.6±9 and 0 ± 0, respectively. Atmospheric concentrations of mercury vapors (µg/m^3) in dental clinics and urinary concentration of this toxic metal (µg/l) in dentists were found to be 4.11±2.34 and 4±3, respectively. Although these values were below the recommended TLV and BEI for this compound, they were significantly higher than those of referent group. Similarly, analysis of the data revealed that dentists had a significantly higher prevalence of symptoms and signs such as hyperpigmentation, respiratory disorders, erethism, anxiety, insomnia, vague fears and loss of memory, than their control counterparts. While these differences could not necessarily causally be linked with mercury exposure, the fact that similar toxic effects are known to be associated with this chemical indicates that occupational exposure to this toxic agent, even at low levels, should be prevented or minimized.

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The gill, kidney and liver DNA integrity of Liza aurata in the seasonal assessment of a costal lagoon (Ria de Aveiro, Portugal) pollution

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Aims: Among the several pernicious effects of xenobiotics, DNA damage is of particular concern because it may cause adverse heritable effects as well as potential diseases. Some of the most common DNA lesions are single strand breaks which are sensitive biomarkers of genotoxicity and have been classified as potentially pre-mutagenic lesions. Thus, the objective of this study was to seasonally (Spring, Summer, Autumn and Winter) assess L. aurata DNA integrity on three different tissues (gill, kidney and liver), in a coastal lagoon (Ria de Aveiro, Portugal) that over several decades had received industrial, agricultural and domestic discharges.

Methods: Study Area: The Ria de Aveiro is a shallow estuary that is permanently connected to the sea. The sampling sites were selected on a geographic distribution basis along the main channels taking into account the various types and sources of contamination as well as the selection of a (theoretically) unpolluted reference point. The sampling sites were: Torreira (TOR), an intermediate region of the longest channel (S. Jacinto-Ovar channel), far from the main polluting sources and thus assumed as the reference site; Barra (BAR), the initial part of the Mira channel close to the lagoon entrance and subject to a considerable naval traffic; Gafanha (GAF) situated in the vicinity of a deep-sea fishing port and dry-docks, also connected with the main channel coming from Aveiro city carrying domestic discharges; Rio Novo do Principe (RIO), located at the terminal area of the Vouga River, 6.5 km distant from a pulp/paper mill effluent outlet, that discharged to this water course during nearly five decades (until the year 2000); Laranjo (LAR), close to a chlor-alkali plant (6 km), an important source of metal contamination (mainly HgCl2); Vagos (VAG), located at the terminal part of the Ílhavo channel, receiving municipal and domestic effluents with high levels of PAHs. Sampling: Golden grey mullet (Liza aurata) was caught in the Spring, Summer, Autumn and Winter of 2006, during low tide using a traditional beach-seine net named “chincha”. Juvenile specimens were selected on the basis of their size. Biometrical parameters, such as fish weight and total length ranged from 18.71 - 41.96 g and 12.9 - 16.5 cm, respectively.

DNA alkaline unwinding assay: Deep frozen portions of blood were submitted to DNA isolation procedure (Genomic DNA Purification Kit, Fermentas). DNA integrity measurements were performed according to Rao et al. (1996) as previously adapted by Maria et al. (2002). DNA single and double strands were separated by hydroxyapatite (20%) ‘‘batch’’ elution and quantified with a DNA-specific fluorescent dye (2.5 nM bisbenzimide), after alkaline unwinding. Data from DNA unwinding technique were expressed as an F-value [DNA integrity (%)], determined by applying the following equation: F = (ds/(ds+ ss)) x 100 where ss is the relative fluorescence of the single-stranded eluent of a sample minus the single-stranded control blank fluorescence value, and ds is the relative fluorescence of the corrected double stranded eluent of the same sample.

Integrated biomarker response (IBR): A method for combining all the measured biomarker responses into one general ‘‘stress index’’ termed ‘‘Integrated Biomarker Response’’ (IBR), (Beliaeff and Burgeot, 2002; Broeg and Lehtonen, 2006). Statistical analysis: Results are expressed as means ± SE (standard error) corresponding to experimental groups of six fish (n=6). A statistical data analysis was done using Statistica software (StatSoft, Inc., Tulsa, OK). The assumptions of normality and homogeneity of data were verified. A factorial analysis of variance (Two-Way ANOVA) was performed in order to assess significant effects in each site and the differences between seasons; this analysis was followed by post-hoc Fisher LSD test to signal significant differences between groups (Zar, 1999). The significance of the results was ascertained at $\alpha = 0.05$.

Results: In gill, DNA integrity was significantly higher at BAR and VAG (Spring) hwn compared to the reference site (TOR). However, the gill DNA integrity was significantly lower at BAR, GAF, RIO and LAR in Autumn.

Kidney DNA integrity was significantly lower than control at BAR (Spring) and GAF (Winter). However, in Spring DNA integrity was significantly higher than control at GAF and VAG whereas in Summer kidney DNA integrity was higher at GAF, RIO, LAR. Moreover, in Autumn DNA integrity was found higher at BAR. However, in Winter DNA integrity found lower in fish caught at GAF.

Liver DNA integrity was significantly higher than TOR at VAG in Spring and significantly lower in Autumn at GAF, RIO, LAR and VAG.

In the studied organs, differences were found between seasons in all the studied sites (including control).
TOR displayed the lowest IBR values in Spring, Summer and Autumn. However, in Winter, the lowest IBR value was found in BAR. The highest IBR values (most affected sites) were found in VAG (Spring), GAF (Summer and Winter) and BAR (Autumn).
Conclusions: The present data signals the existence of genotoxicants in the Aveiro Lagoon. The observed tissue differences suggest that gill is being subject to more genetic damage than liver and kidney. The integrated biomarker response index signals VAG, GAF, BAR and GAF as the most affected sites in Spring, Summer, Autumn and Winter, respectively. The effectiveness of cellular defences associated with the abiotic factors such as temperature, hydrodynamics, salinity among other factors that may change the xenobiotics bioavailability, interfere with waterborne pollutants genotoxicity.

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Thyroid hormones and stress responses in field monitoring - an integrated biomarker approach

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Aims: Hypothalamo-pituitary-thyroid (HPT) and hypothalamo-pituitary-interrenal (HPI) axes play a central role in a wide range of important homeostatic mechanisms in fish. Changes on thyroid hormones and cortisol as well as on glucose and lactate levels can reflect endocrine alterations, reducing fish physiological competence and possibly survivorship. Thus, the objective of this study was to assess the thyroid-stimulating-hormone (TSH), the thyroid hormones (triiodothyronine - T3 and free thyroxine T4) and stress (cortisol, glucose and lactate) responses, in Liza aurata caught at a coastal lagoon (Ria de Aveiro) that presents different contamination profiles.
Methods: Study Area: The Ria de Aveiro is a shallow estuary that is permanently connected to the ocean through a narrow channel 1.3 km long, 350 m wide and 20 m deep. The estuary is about 45 km long (NNE-SSW) and 8.5 km wide, and the wetland area covers approximately 83 (high tide) to 66 km2 (low tide). The hydrological circulation is dominated by the water exchange with the ocean due to the low freshwater input compared with the seawater penetrating the system. As a consequence of the low freshwater discharge from the tributaries and the diffuse runoff combined with the tidal penetration, the Ria de Aveiro exhibits a spatial gradient of salinity from about 0 in the upper branches to about 36 at the bar entrance. The sampling sites were selected on a geographic distribution basis along the main channels taking into account the various types and sources of contamination as well as the selection of a (theoretically) unpolluted reference point. The sampling sites were: Torreira (TOR), an intermediate region of the longest channel (S. Jacinto-Ovar channel), far from the main polluting sources and thus assumed as the reference site; Barra (BAR), the initial part of the Mira channel close to the lagoon entrance and subject to a considerable naval traffic; Gafanha (GAF) situated in the vicinity of a deep-sea fishing port and dry-docks, also connected with the main channel coming from Aveiro city carrying domestic discharges; Rio Novo do Principe (RIO), located at the terminal area of the Vouga River, 6.5 km distant from a pulp/paper mill effluent outlet, that discharged to this water course during nearly five decades (until the year 2000); Laranjo (LAR), close to a chlor-alkali plant (6 km), an important source of metal contamination (mainly HgCl2); Vagos (VAG), located at the terminal part of the Ílhavo channel, receiving municipal and domestic effluents with high levels of PAHs.
Sampling: Golden grey mullet (Liza aurata) was caught in the Autumn 2005, during low tide using a traditional beach-seine net named “chincha”. Juvenile specimens were selected on the basis of their size. Biometrical parameters, such as fish weight and total length ranged from 18.71 - 41.96 g and 12.9 - 16.5 cm, respectively. Blood was collected from the posterior cardinal vein using a heparinized Pasteur pipette and plasma separation performed on Eppendorf centrifuge (14 000 rpm).
Hormonal responses measurement: The determinations of cortisol, TSH, T3 and T4 were performed in plasma, using diagnostic ELISA direct immunoenzymatic kits (Diametra, Italy). The absorbance was measured at 450 nm in a microplate reader (ASYS Hitech). The cortisol in the sample competes with horseradish peroxidase (HRP)-cortisol for binding onto the limited number of anti-cortisol sites in the microplate wells. The enzyme substrate (H2O2) and the TMB (3,3´, 5,5´ tetramethylbenzidine)-

substrate are added, and after an appropriate time has elapsed for maximum color development, the enzyme reaction is stopped and the absorbances are determined. Cortisol concentration in the sample is calculated based on a series of standards and the color intensity is inversely proportional to the cortisol concentration in the sample. The methods for free T3 and free T4 follow the same principles of the cortisol test, requiring immobilized T3 or T4 antibodies, as well as HRP-T3 or HRP-T4 conjugates. Concerning TSH, an antibody specific to the h-chain of TSH molecule is immobilized on microwell plates and other antibodies to the TSH molecule are conjugated with HRP. TSH from the sample is bound to the plates. The enzymatic reaction is proportional to the amount of TSH in the sample.
Intermediary metabolism responses measurement: Plasma glucose was measured spectrophotometrically (340 nm) according to the method modified from Banauch et al. (1975), based on the quantification of NADH after a glucose oxidation catalysed by the glucose-dehydrogenase. The quantity of NADH formed is proportional to the glucose concentration. Plasma lactate levels were determined spectrophotometrically (340 nm) according to the method modified from Noll (1974) using LDH, ALT and NAD, measuring the NADH appearance.
Integrated biomarker response (IBR): A method for combining all the measured biomarker (with the exception of T4, since no sample was available to perform this parameter in LAR) responses into one general ‘‘stress index’’ termed ‘‘Integrated Biomarker Response’’ (IBR), (Beliaeff and Burgeot, 2002; Broeg and Lehtonen, 2006). Statistical analysis: Results are expressed as means ± SE (standard error) corresponding to experimental groups of six fish (n=6). A statistical data analysis was done using Statistica software (StatSoft, Inc., Tulsa, OK). The assumptions of normality and homogeneity of data were verified. A factorial analysis of variance (Two-Way ANOVA) was performed in order to assess significant effects in each site and the differences between seasons; this analysis was followed by post-hoc Fisher LSD test to signal significant differences between groups (Zar, 1999). The significance of the results was ascertained at $\alpha = 0.05$.
Results: TSH levels of fish caught in the polluted sites were significantly higher than control site (TOR) at LAR. However, at RIO, LAR and VAG free T3 levels were significantly lower than TOR. This pattern of responses was not followed by free T4 levels which, at BAR, were significantly lower. In terms of stress responses, cortisol was significantly lower than TOR at VAG whereas glucose and lactate were significantly higher in LAR. The integrated biomarker response signalled LAR and VAG as the most affected sites and GAF as the site were these biomarkers were least affect.
Conclusions: Considering the results of this study, it can be suggested that fish populations living in the current studied areas can suffer impairment of several biological functions. According to the IBR index, LAR and VAG are the most affected sites, though these biomarkers have also signalled other sites as potential hazardous to L. aurata.

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Studying the possible linkage between 8-hydroxy-2’-deoxyguanosine, DNA integrity and erythrocytic nuclear abnormalities in Liza aurata and its utility in biomonitoring a coastal lagoon

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Aims: The oxidation of guanine in the C8 position results in the formation of 8-hydroxy-2’-deoxyguanosine (8-OHdG), one of the predominant and most studied oxidative free radical induced lesions of DNA which may result in site specific mutagenesis. Although the consequences of 8-OHdG base modification for cell integrity are not completely known, in the absence of base modification repair such impaired DNA may lead to strand misreading, mutations, altered gene expression, strand breaks and cytotoxicity. The different frequencies of nuclear anomalies may be related to specific genotoxic events associated to the different mechanisms of action of the carcinogenic/mutagenic agents. However, the mechanisms for nuclear abnormalities formation are not completely understood. Thus, the objective of this study was to assess the possible linkage between these damages to DNA. Moreover, we aimed to assess seasonally, the environmental pollution of a coastal lagoon (Ria de Aveiro) in terms of genotoxic hazard to fish using these biomarkers. For this purpose, an integrated biomarker response index was used.
Methods: Study Area: The Ria de Aveiro is a shallow estuary that is permanently connected to the

ocean. The sampling sites were selected on a geographic distribution basis along the main channels taking into account the various types and sources of contamination as well as the selection of a (theoretically) unpolluted reference point. The sampling sites were: Torreira (TOR), an intermediate region of the longest channel (S. Jacinto-Ovar channel), far from the main polluting sources and thus assumed as the reference site; Barra (BAR), the initial part of the Mira channel close to the lagoon entrance and subject to a considerable naval traffic; Gafanha (GAF) situated in the vicinity of a deep-sea fishing port and dry-docks, also connected with the main channel coming from Aveiro city carrying domestic discharges; Rio Novo do Principe (RIO), located at the terminal area of the Vouga River, 6.5 km distant from a pulp/paper mill effluent outlet, that discharged to this water course during nearly five decades (until the year 2000); Laranjo (LAR), close to a chlor-alkali plant (6 km), an important source of metal contamination (mainly $HgCl_2$); Vagos (VAG), located at the terminal part of the Ílhavo channel, receiving municipal and domestic effluents with high levels of PAHs. Sampling: Golden grey mullet (Liza aurata) was caught in the Spring, Summer, Autumn and Winter of 2006, during low tide using a traditional beach-seine net named “chincha”. Juvenile specimens were selected on the basis of their size. Blood was collected from the posterior cardinal vein using a heparinized Pasteur pipette and used for smear preparation and submitted to DNA isolation procedure, as well as plasma separation using an Eppendorf centrifuge (14 000 rpm). 8-hydroxy-2’-deoxyguanosine (8-OHdG) assay: 8-hydroxy-2´-deoxyguanosine (8-OHdG) was quantified in plasma through a competitive enzyme-linked immunosorbent assay using an IBL (Hamburg) kit. DNA alkaline unwinding assay: Deep frozen portions of blood were submitted to DNA isolation procedure (Genomic DNA Purification Kit, Fermentas). DNA integrity measurements were performed according to Rao et al. (1996) as previously adapted by Maria et al. (2002). DNA single and double strands were separated by hydroxyapatite (20%) ‘‘batch’’ elution and quantified with a DNA-specific fluorescent dye (2.5 nM bisbenzimide), after alkaline unwinding. Data from DNA unwinding technique were expressed as an F-value [DNA integrity (%)], determined by applying the following equation: $F = (ds/(ds + ss)) \times 100$ where ss is the relative fluorescence of the single-stranded eluent of a sample minus the single-stranded control blank fluorescence value, and ds is the relative fluorescence of the corrected double stranded eluent of the same sample. Erythrocytic nuclear abnormalities (ENA) assay: Blood smears were fixed with methanol during 10 minutes and stained with Giemsa (5%) during 30 minutes. The nuclear abnormalities were scored in 1000 mature erythrocytes sample per fish, according to the criteria of Schmid (1976), Carrasco et al. (1990) and Smith (1990), adapted by Pacheco and Santos (1996). The final result was expressed as the mean value (‰) of the sum for all the individual lesions observed. Integrated biomarker response (IBR): A method for combining all the measured biomarker responses into one general ‘‘stress index’’ termed ‘‘Integrated Biomarker Response’’ (IBR), (Beliaeff and Burgeot, 2002; Broeg and Lehtonen, 2006). Statistical analysis: Results are expressed as means ± SE (standard error) corresponding to experimental groups of six fish (n=6). A statistical data analysis was done using Statistica software (StatSoft, Inc., Tulsa, OK). The assumptions of normality and homogeneity of data were verified. A factorial analysis of variance (Two-Way ANOVA) was performed in order to assess significant effects in each site and the differences between seasons; this analysis was followed by post-hoc Fisher LSD test to signal significant differences between groups (Zar, 1999). The significance of the results was ascertained at $\alpha = 0.05$.

Results: In the present study, there is a clear indication that the L. aurata DNA is suffering oxidative damage, measured as 8-OHdG. Thus, in Spring, three sites (GAF, LAR and VAG) showed significantly higher levels of this oxidative damage product. However, these results were not observed in Summer, where no significant differences were observed when compared to the control site (TOR). In Autumn the fish captured at RIO, LAR and VAG showed significantly higher levels of 8-OHdG whereas in Winter oxidative DNA damage was observed in BAR, RIO, LAR and VAG. Comparing the sites seasonally, we found significant differences in the control site. 8 OHdG levels in Summer were significantly lower than in Spring and Winter. Moreover, significant differences were also observed in contaminated sites. In the duration of the study, the highest 8-OHdG levels were found in Spring at GAF, LAR and VAG. On the other hand, the lowest 8 O-HdG levels were found in Summer. In terms of DNA strand breaks, DNA integrity was significantly decreased in Spring at BAR, RIO and LAR. However, in Summer and Autumn DNA integrity was significantly increased at BAR and VAG. No significant changes in the integrity of the DNA were found in Winter. Significant differences were found in the control site during the year. Thus, in Spring DNA integrity was found significantly higher when compared with the other seasons. However, this higher integrity in Spring was not found for the other studied sites. ENA, that can be considered at a higher biological level in relation to 8-OHdG and thus more relevant in terms of potential effect to the species, signalled VAG as a site having the higher levels of genotoxicants. Thus, in Spring ENA frequency was significantly higher at LAR and VAG. However, in the following seasons, ENA levels were only found higher at VAG (Summer and Winter). On the other hand, at BAR, GAF and RIO (Summer) and LAR (Autumn) ENA frequency was significantly decreased when compared to control site. No seasonal differences were found neither in

the control site nor in RIO though found in other sites. The IBR index signalled LAR (Spring), VAG (Summer and Winter) and BAR (Autumn) as the most affected sites (highest IBR) were
Conclusions: The Aveiro Lagoon is subject to genotoxic pressure of xenobiotics that had been released in this aquatic system for more than 50 years. However, the oxidative damage seems to be influenced by the season, which may also be related with abiotic factors like rain that, through agricultural fields runoff, sediments resuspension and temperature interfere with the chemicals bioavailability. Though 8-OHdG signalled the existence of DNA oxidation, cytogenetic damage was found in fewer sites, suggesting that L. aurata DNA repair mechanisms were being effective, not allowing a straightforward linkage between the studied parameters. These results emphasize the importance of seasonal studies when biomonitoring coastal lagoons.

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The effect of nutrient concentrations in Thermaikos Gulf (North Aegean Sea, Greece) on the uptake of organic pollutants by the mussel Mytilus galloprovincialis

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Aims: The scope of this study is to:
a) evaluate the chemical quality of seawater along the coastline of Thermaikos Gulf based on nutrient data and the stoichiometric nutrient balance;
b) to study the bioaccumulation of organic pollutants (hydrocarbons, DDTs, PCBs) by the mussel Mytilus galloprovincialis;
c) to examine the importance of nutrient status of the sea environment to the uptake of organic pollutants by mussels
Introduction: Thermaikos Gulf, in the northwestern part of the Aegean Sea, host the most extended and productive mussel aquacultures of Greece (70% of the whole production). The coastal are of the gulf receives urban effluents by Thessaloniki City (more than 1,200,000 population) and the discharges of the watersheds of Axios, Aliakmon, Loudias and Gallikos Rivers. Until recently, the sewage of Thesaloniki City was discharged untreated in the inner Thermaikos Gulf. In addition, industrial effluents drain into the gulf. This anthropogenic pressure had changed the trophic status of Thermaikos Gulf into a eutrophic one High nitrate and phosphate levels have stimulated algal blooms in Thermaikos Gulf, some of which were toxic or harmful. It is known that the mussel Mytilus galloprovincialis is used as a bioindicator of the degree and extend of chemical pollution. The question is if nutrient limitation is related to the organic pollutants uptake and their accumulation in mussels tissues.
Methods: During the period from August 1997 to December 2006, water and mussel samples were collected at seven coastal stations in Thermaikos Gulf which were chosen in order to take into account the different anthropogenic pressures in the area, such as mussels farms (stations X and AG), touristic activities and sewage discharges (stations MH, AT, P), agricultural activity (station L), atmospheric inputs from the airport of Thessaloniki (station A).
Water samples were collected at different standard depths using NISKIN bottles. Ammonium analysis was performed with a Perkin Elmer UV/VIS (Lambda 20) spectrophotometer, whereas phosphate, silicate, nitrite and nitrate were measured with a BRAN+LUEBBE nutrient autoanalyser III.
The mussels were collected form the seven locations by diving. For hydrocarbon analysis freeze dried tissue was spiked with known quantities of internal standards and and saponified with methanolic KOH. The hydrocarbons were extracted with n-hexane, cleaned-up and fractionated on silica gel columns and determined by gas chromatography-mass spectrometry. The compounds determined included aliphatic hydrocarbons, and polycyclic aromatic ones with 2-6 aromatic rings. For the organochlorine analysis the freeze-dried material was Soxhlet extracted for 24 hrs with a mixture of dichloromethane-pentane 1:1, the extracts were cleaned-up and fractionated on deactivated alumina columns and the final determination was performed on a gas chromatograph equipped with an ECD detector. The compounds determined included HCB, lindane, p,p’-DDT and its metabolites (p,p’-DDE and p,p’-DDD) and eleven PCB congeners.
Results: The mean integrated values of inorganic nitrogen for the period 1997 - 2006 in water column showed high levels at the station X close to the mussel cultures at the western part of the gulf, as well as at station MH, at the eastern part of the gulf, which is influenced by sewage discharges. It is

noteworthy, that the mean N:P stoichiometric ratio at all the stations was lower than the theoretical value 16:1, except from station MH, where the mean ratio value was higher. Station X showed the highest phosphate and nitrate concentrations (3.500 μmol/L for phosphate during October 2000, 8.88 μmol/L for nitrate during April 1998), followed by the MH station with relatively high nitrate concentration (6.22 μmol/L during March 1998) and the highest ammonium concentrations (11.13 μmol/L during September 2000).

In 1997, station AT at the eastern part of the gulf showed high ammonium values. After October 2000 a significant decrease of inorganic nitrogen was observed at all stations showed significantly lower N loads especially for ammonium During the period from September 1997 to September 2000 high values of nitrogen were recorded leading to high stoichiometric N:P ratio and phosphorus seems to be the limiting factor for the growth of phytoplankton, which may initially increase food quantity and quality. On the contrary, low N:P ratio values were calculated during the period October 2000 - December 2006 at almost all the selected stations, indicating that during this period the trophic status of the area showed a shift from likelihood P-limitation to N-limitation.

Relatively high aliphatic hydrocarbon concentrations (>100 μg/g) were measured in 1997 and 1998 especially in the mussels collected from natural populations at the eastern part of the gulf (Stations P, AT). After 2000, when decreased N loads were observed, aliphatic values were also significantly lower, while during this period no differentiation was observed between the natural populations at the east part of the gulf and the mussel farms at the western part. PAHs followed a similar pattern with that of aliphatics. Very high values (>1000 ng/g) were measured at the eastern part of the gulf before 2000, while during 2000-2006 in all stations the PAH concentrations were well below 400 ng/g with a homogeneous spatial distribution. In all cases mussels preferentially bioaccumulated PAH compounds with lower MW and higher water solubility and especially phenanthrene and its methylated analogs known to be of petrogenic origin. Benzo(a)pyrene, a known carcinogen, was detected in all stations and periods in very low concentrations, well below the limits considered as dangerous for human consumption. DDTs concentrations were generally low (<40 ng/g) but again the highest values were recorded before 2000 at the eastern part of the gulf. In most cases p,p-DDE was the predominant component of the DDTs, but in some cases and especially at station AT unexpectedly high percentages of the parent DDT compound were recorded and this might indicate possible recent inputs of DDT in the gulf PCBs concentrations were very low in all cases and clearly lower than those considered as dangerous for the human health. In contrast with the other organic pollutants no differentiation was observed among the stations and the sampling periods. The hexachloro- 153 and 138 were always the dominant congeners followed by the pentachloro- 118 and 101.

Conclusions: Mean integrated concentrations of nutrients in seawater as well as the organic pollutants in mussels showed similar spatial and temporal variations.

During the period 1997 to September 2000, the concentrations of inorganic nitrogen in seawater were significantly higher than those recorded after October 2000. A shift from P- limitation to N- limitation was observed, which was followed by the significant decrease of organic pollutants in the tissues of the mussels.

In all stations and periods organic pollutant concentrations in the mussel tissues were clearly below the limits considered as dangerous for human health.

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Cadmium is a good indicator of exposure to environmental tobacco smoke in children

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Background: Environmental tobacco smoke (ETS) is a major source of indoor air contaminants. Expose to ETS is an important predictor for heavy metal exposure, especially of cadmium (Cd). ETS from parental smoking may cause many respiratory disorders in children, particularly in infants.

Objective: To determine whether hair cadmium levels reflects exposure to ETS and correlation between hair Cd levels, wheezing and respiratory truck infection epizodes.

Methods: This study was performed in the outpatient clinic of Pediatric Allergy Department in

Keçiören Education and Training Hospital. 61 children with recurrent wheezing (RW) between the ages of 1 and 6 years and 34 age-mached healthy children (HC) were enrolled in to the study. The two groups were compared in the mean of hair cadmium levels and history of exposure to ETS at home.
Results: The history of smoking at home (62.3% vs 34.3, p: 0.018) and hair Cd levels (29.61±3.25 vs 16.79±2.76, p:0.009) were significantly higher in RW group than HC group. Hair Cd levels were closely corelated with number of smokers at home (r: 0.859, p<0.001), number of wheezing and upper respiratory infection (r: 0.346, p:0.006 and r:0.339, p:0.009, respectively) in the last year in RW group. Additionally number of smokers at home was significantly related with number of wheezing and upper respiratory infection (r: 0.442, p<0.001 and r:0.420, p<0.01, respectively) in the last year in RW group. However, hair Cd levels were foun to be significantly corelated with number of smokers at home (r:0.671, p<0.001) in HC group but nor the number of smokers at home nor the hair Cd levels were found to be coraleted with upper respiratory infection in the last year in HC group (r:0.196 p:0.267 and r:0.217 p:0.219 respectively).
Conclusion: Our results demonstrated that hair Cd is a good indicator of exposure to ETS at home in healthy children and in children with recurrent wheezing. But the children with RW are much more susceptible to exposure to ETS than healthy children and exposure to ETS at home in children with RW is a significant trigger of wheezing and respiratory infections.

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Involvement of midkine and galectin-3 in cadmium induced cardiomyopathy

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Accumulation of the wide spread environmental toxin cadmium (Cd) in tissues results in toxicity. Heart is one of the most effected tissues. Cd exposure induces inflammation in effected tissues.
The present study was focused to evaluate roles of TNF-α during Cd toxicity and their relationships with midkine and galectin-3 levels. Male Wistar rats were exposed to Cd at the dose of 15 ppm for 8 weeks. Inflammatory status in hearts was evaluated with measurement of tissue TNF-α levels. Histopathological examination of heart was carried out by light microscopy. Heart tissue caspase-3 level was used to identify apoptosis. Tissue midkine and galectin-3 levels were evaluated by ELISA. Heart sizes were increased after Cd toxicity. A significant increase in galectin-3, midkine tissue levels was seen after Cd toxicity, this was accompanied with a significant increase in the TNF-α levels. Histopathological examination under light microscope suggested a combination of ongoing necrosis and apoptosis. Increased caspase-3 levels were measured after Cd toxicity.
Chronic Cd administration induces inflammation and apoptosis in rat hearts. Cd causes increased midkine and galectin-3 production from heart tissue. The formation of TNF-α due to Cd exposure may likely trigger this mechanism.

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Photo-fenton treatment of a carbamate pesticide, pirimicarb, in aqueous solutions

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Pesticide polluted water and wastewaters constitute one of the major environmental concerns and demand efficient and operative treatment methods in order to cope with the continuously tighter regulations imposed about the admissible pesticides concentrations.

Pirimicarb (2-dimethylamino-5,6-dimethylpyrimidin-4-yl dimethylcarbamate) is a fast-acting carbamate insecticide used to control aphids in a great variety of crops including cereals, sugar beet, potatoes, fruit, vegetables. It is very selective, with quick knockdown effect and it is considerd to be ideal for use in greenhouses.

In this study, the degradation of pirimicarb in aqueous solutions using the homogeneous photocatalytic method based on the photo-Fenton reaction was investigated. During the photo_Fenton reaction, which is the combination of the well-known Fenton reaction (Fe2+/H2O2) with UV irradiation, hydroxyl radicals are formed, while Fe2+ ions are oxidized and reduced by means of UV light turning the process to catalytic.

The main objectives of the study were: I) to evaluate the kinetics of the pesticide disappearance in aqueous media of different composition such as river, lake and deionized water, II) to examine the influence of various parameters such as initial concentration of pesticide, iron or hydrogen peroxide, pH value and presence of different inorganic anions (derived from the salt used as source of iron), III) to evaluate the degree of mineralization, IV) to assess the detoxification efficiency of the studied processes.

A 500mL pyrex UV reactor equipped with a 125W high-pressure mercury lamp surrounded by a pyrex filter blocking wavelengths below 290nm was used for the photocatalytic experiments. Pirimicarb concentration was measured by a Shimadzu 10AD liquid chromatograph, the Dissolved Organic Carbon (DOC) was determined by a Shimadzu V-csh TOC analyzer and for the examination of the toxic properties of the samples collected at specific time intervals throughout the photocatalytic treatment, measurements were carried out using the luminescent bacteria V. ficheri and a MICROTOX test.

It has been found that photo-Fenton assisted reaction follows pseudo-first order kinetics when the degradation of pirimicarb is concerned. Increased iron's and oxidant's concentration ameliorate the process, but, due to the catalytic character achieved thanks to UV irradiation, the concentrations used are significantly small, serving that way the goal of diminishing reactants' consumption and turning the technique to financially viable. Inorganic ions strongly affect the oxidation rate, a fact that could be attributed to the complexation of some anions with iron ions or to hydroxyl radicals scavenging caused by certain anions.

Prolonged illumination (6 hours), lead to mineralization up to almost 90%. The toxicity of the treated solution was evaluated, in order to compare the acute toxicity of pirimicarb and its photoproducts. The detoxification efficiency although it did not follow the rate of pesticide disappearance, it took place in considerable extent.

Photo-Fenton reaction is considered a promising treatment method that can be applied to a wide range of pollutants and provides the important possibility of using solar light as irradiation source, reducing that way the cost and the energy outlay.

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Preparation and characterization of activated carbon made from peach stone, palm fruit shell, cashew nut shell, coconut shell and bituminous coal, and testing their performance in the Catalytic Wet Peroxide Oxidation (CWPO) of maleic acid

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Preparation, performance and characterization of activated carbons from bituminous coal, coconut shells, palm fruit shells, peach stones and cashew nut shells were investigated. Preparation and performance test were done at the University of Dar es Salaam while characterization was done at Brown University, Rhode Island, USA. Physical activation of pyrolised char with carbon dioxide was done at 900℃, 30 minutes holding time and heating rate of 20℃/min under nitrogen flowrate of 150 cm^3/min. Under these conditions, the activated carbons yielded the maximum Brunauer Emmett-Teller (BET) surface areas of 101, 351, 389, 428 and 692 m^2/g for bituminous coal, coconut shells, palm fruit shells, peach stones and cashew nut shells respectively. The morphology structures of the samples were determined using a Scanning electron microscopy (SEM), while thermal stabilities were determined using thermal gravimetric analysis (TGA).The performance of these activated carbons were tested in catalytic wet peroxide oxidation (CWPO) of maleic acid (0.01 g/l), 0.5 g/l of activated carbons at low temperature and pressure, 50℃ and 1 .2 Pa respectively. Finally, it can be concluded that activated carbon (AC) catalysts used in this study are effective for liquid phase oxidation of organic compounds and can be applied for catalytic wastewater treatment systems.

Key words: Activated Carbon, Catalytic Wet Peroxide Oxidation, Activation, Microporosity, Surface areas

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The influences of the nuclear power plant temelin on the Vltava River

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Aims: The Vltava River (in German: Moldau), beeing 430 km long, is the longest river in the Czech Republic merging with the Elbe. The methodology to analyze the influence of the Nuclear Power Plant Temelín (NPP Temelín) in the South Bohemia on the Vltava River is described and the obtained results summarized.

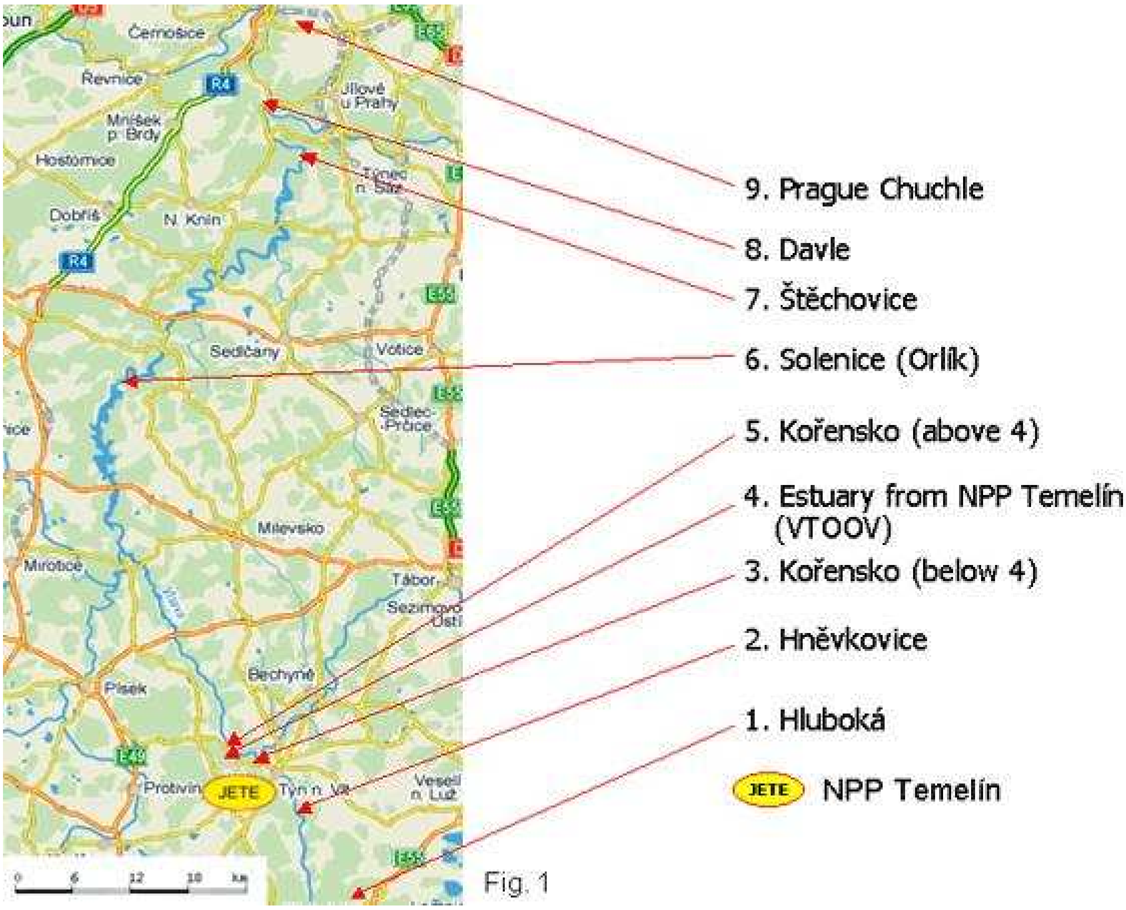
Methods: Using selected sites in the Vltava River from the NPP Temelín to Prague (Fig.1), totally 257 water samples collected in 33 series from February 2005 to September 2007 were analyzed. For each sample, an aliquot of 6mL water was mixed with 10mL of liquid scintillator Rotiszint eco plus (Karlsruhe, Germany) and the tritium volume activity, including standard deviation, determined by a liquid scintillation spectrometer using setting for low-levels (Tri-Carb 2900 TR, Perkin-Elmer, USA). The measured values were correlated with concentrations of cations in aliquots taken from the same samples and analyzed by inductively coupled plasma mass spectrometry ICP-MS (PQ Ex-Cell, Thermo, England). Totally, concentrations of 32 different cations were determined. Finally, the corresponding concentrations of 8 anions were also determined (Flow Injection Analyzer, Tecator, Sweden). For considering of the total concentration of ions, conductivity of all samples was measured. All the above given values were considered in relation to those from the estuary of the damping object of waste waters of the Nuclear Power Plant Temelín (sign.VTOOV). The corresponding values of water from the river collected above this estuary (Fig.1, the vicinity of VTOOV including the dam Kořensko see Fig.2) were analyzed for comparison.

Results: An example of analyses is given in Fig.3. It is evident that the tritium volume activity is negligible in the river water except the sample collected near below VTOOV due to the relatively high value in VTOOV.

The tritium volume activity was found lower than 10 Bq/L almost in all measured river samples, only 14 of them had higher activities. Therefore, according to the classification of water purity (the Czech norm ČSN 75 7221 "Classification of surface waters in accordance with their qualities"), by the 3H-analysis in 243 samples of water from the Vltava River was found the highest purity (classification No.1), only 14 samples had classification No.2.

However, it was proved that concentrations of cations and/or anions in some samples somewhat deteriorated the quality of water (e.g., ^{31}P,^{54}Fe,^{65}Cu,^{66}Zn). In contradistincion to the courses given in Fig.3, no general correlation between tritium volume activities and concentrations of ions was found. These evaluations have been in progress now and they will be published elsewhere.

Conclusions: It was proved that for a relatively small deterioration in water quality in the Vltava River below the Nuclear Power Plant Temelín some cations and/or anions are responsible while the volume activity of tritium was found lower than 10 Bq/L excepting several samples (5% of all analyzed samples, especially, near below VTOOV).



[1]

Fig.1. The collection sites of water from the Vltava River.

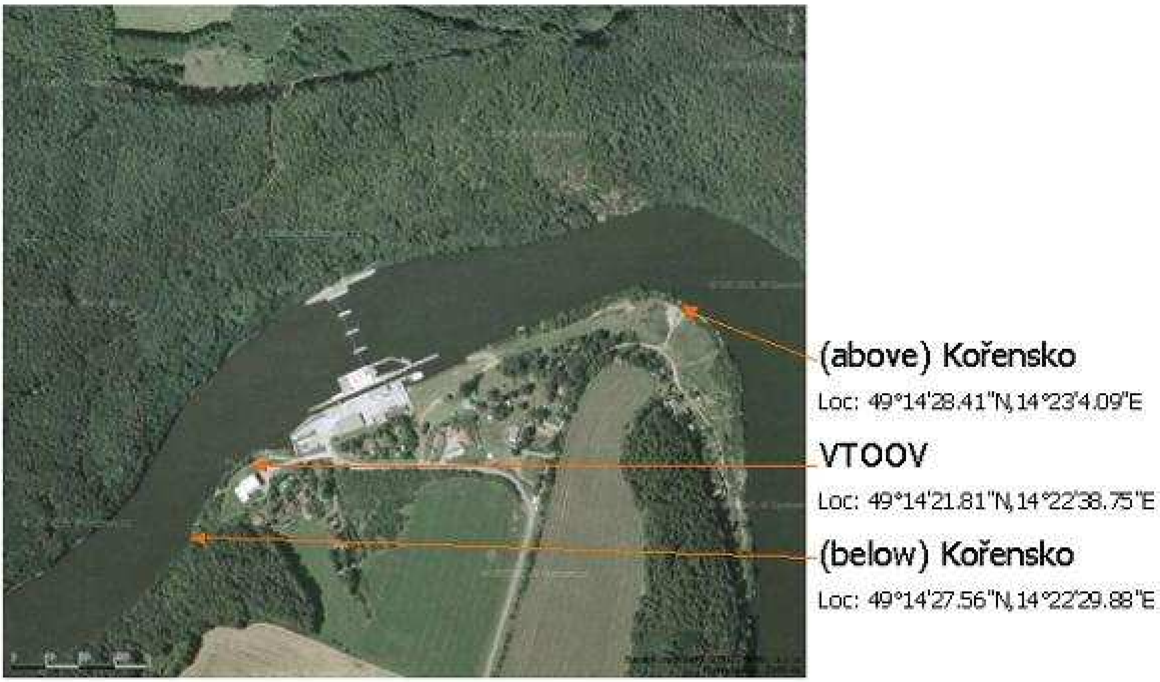


Fig. 2

[2]

Fig.2. The close vicinity of the estuary from the damping object of waste waters of NPP Temelín (sign.VTOOV).

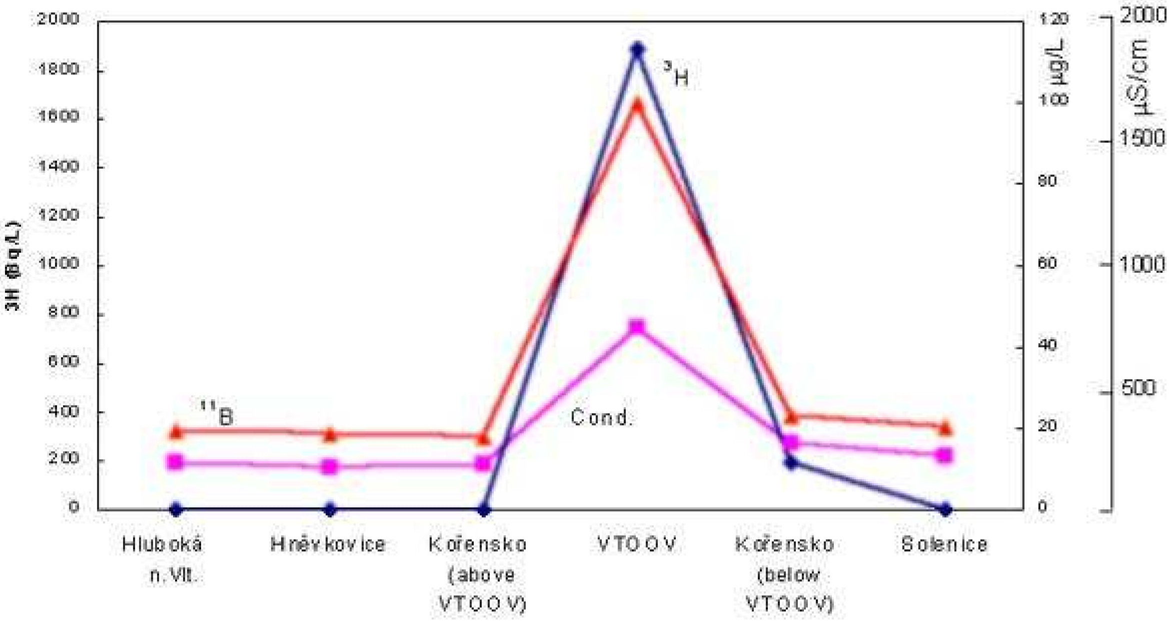


Fig. 3

[3]

Fig.3. Example of analysis of a sample collected February 13,2006.

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Biodegradation of azo dyes by micoorganisms isolated from textile wastewater

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The textile wastewater is a complex mixture of chemicals including high levels of suspended solids and heavy metals, with alkaline pH, discharged at relatively high temperatures.

The organic pollutants are phenolic compounds (toluene, benzene and chlorobenzene) and naphthalene (substituted phenols). These have toxic effects.

Physicochemical treatment based on activated charcoal, ozonation, flocculation and reverse osmosis are used to decolorize the effluent. These methods are costly and biological treatment is more efficient to reduce coloration resulting from azo dyes. In this work, textile wastewater from Ksar Helal (Monastir, Tunisia) produced at 2800 m^3/day wee used. A yeast able to decolorize methyl red in 30 minutes at a rate of 96 % was isolated and studied. Two other reactive azo dyes were tested and biodegraded. The isolate was able to decolorize all of the three dyes with an enzymatic activity of 3.253 U mg-1 of proteins. The enzyme decoloured a mono- and di- azo dyes by fission of azo bond (-N=N-), characteristic chomophore of azo dyes. Optimal conditions of the enzymatic activity were determined (temperature, pH and oxygenation). The SDS-PAGE revealed an area of decolorization in relation with the enzyme.

Finally, the isolated yeast was used to purify neutralised textile wastewater and exhibited a decolorization and dye biodegradation, confirmed by analytical methods (physicochemical and spectroscopic: COD, UV-visible, IR, HPLC and GC/MS). Toxicity tests revealed a non toxic effluent after its treatment with the isolated yeast.

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The adjustment techniques of heavy metals bioaccumulation in the mussel Mytilus galloprovincialis for evaluation of contamination in Moroccan coast

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The marine environment, particularly rich biotope, is characterized at the same time by a remarkable stability of its fundamental properties and a great variability of its microconstituants. However the contribution of metal contaminants via the industrial effluents and the atmosphere, the rivers and their estuaries, can modify the composition of sea water which can become toxic for plants and animals. The direct measure of the contaminants in water is done by sophisticated and expensive analytical techniques which are not easily applicable to many samples taken along coastal. In addition, the temporal variability of coast confers only little significance on a specific measure taken in the water column. This is why moulds it, indicating species of pollution, is largely used in various monitoring and research programs aiming at establishing the temporal and space contaminants variability of the coastal environment.

The purpose of this study is to evaluate the state of contamination by heavy metals in Moroccan coast zones by introducing the condition index (IC : ratio of dry flesh weight to dry shell weight), which makes it possible to adjust the contents of heavy metals measured in the mussel *Mytilus galloprovincialis*. This index represents the biometric variable most strongly correlated with the concentrations of contaminant.

Material and methods:

Sites of taking away

Dar Bouazza is located at approximately 30 km of Casablanca on the Atlantic coast, in the western center of Morocco.

Ouled ghanem is located at 76 km in the south of El Jadida and at 66 km in the north of Safi.

Sampling and pretreatment

The sampling of the marine organisms is done each month between January and September 2007. In the whole of the station, we carry out :

- measure of length, width and thickness of the mussels shells with a slide caliper giving a measure to 1/10 mm ;
- weighing of each individual to 1/1000 g “the total fresh weight”;
- weighing the flesh and the shell “fresh weight”;
- drying at low temperature 42℃ in a stainless drying oven ;
- weighing the flesh and the shell “dry weight”;
- crushing and homogenization of the flesh dries.

Analyze

Acid mineralization is carried out in a microwawe oven (950 W) “Mars 5 CEM) programmed by computer with regulation system of pressure and temperature inside the mineralization bombs. The proportioning of cadmium is carried out by spectrophotometry of atomic absorption (Schimadzu AA 6800), provided with a furnace of graphite and the analysis of zinc is carried out with flame. Mercury is proportioned by spectrophotometry of atomic absorption provided with a cold steam generator (Aula 254).

Results and discussion:

The temporal variations of metal contents in the mussel

The temporal variations of metal contents in the mussel show a seasonal rhythm for the three analyzed elements. Indeed, the maximum concentrations (Cd, Zn and Hg) in mussel tissue at Dar bouazza are recorded in spring with a minimum in summer (Figure 1).

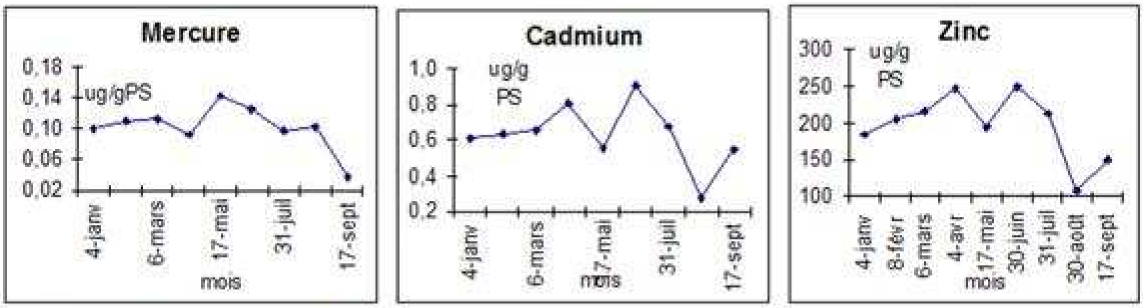


Figure 1: Monthly variation of the concentrations of mercury, cadmium and zinc in mussel tissue at Dar bouazza.

[1]

The temporal variations of the concentrations of mercury, cadmium and zinc in mussel tissue at Ouled ghanem present a seasonal rhythm of bioaccumulation of the metals analyzed in the mussel with a maximum in spring and an estival minimum (figure 2).

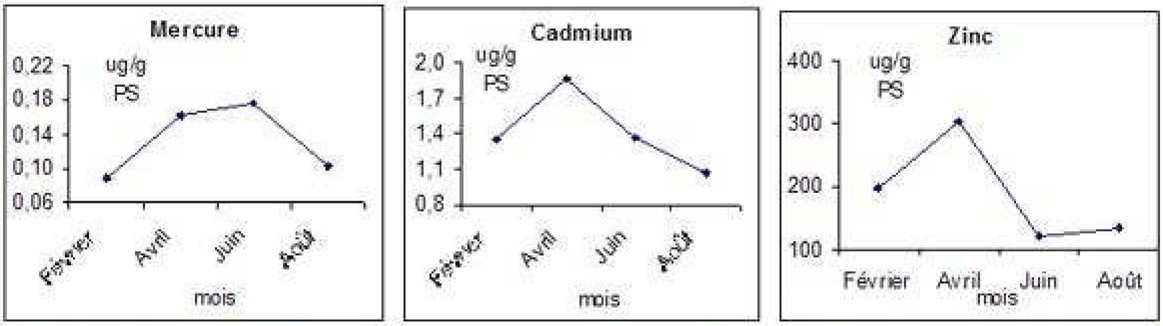
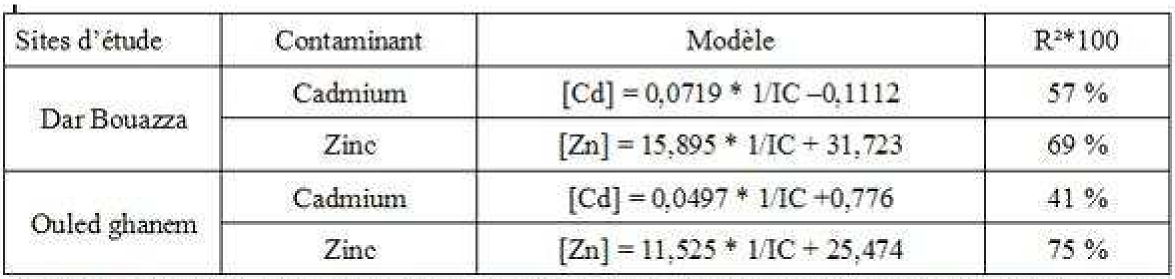


Figure 2: Temporal variation of the concentrations of mercury, cadmium and zinc in mussel tissue at Ouled ghanem.

[2]

Adjustment the rough results

The relation linear, inversely proportional between concentration factor and condition index [ML] = a * 1/IC+b is given in table 1. For mercury no significant relation was recorded between concentration and reverse condition index (R2 very weak).



Sites d'étude	Contaminant	Modèle	R²*100
Dar Bouazza	Cadmium	[Cd] = 0,0719 * 1/IC –0,1112	57 %
	Zinc	[Zn] = 15,895 * 1/IC + 31,723	69 %
Ouled ghanem	Cadmium	[Cd] = 0,0497 * 1/IC +0,776	41 %
	Zinc	[Zn] = 11,525 * 1/IC + 25,474	75 %

Table 1 : Models of regression between the contaminated concentration and reverse condition index with R² is correlation coefficient.

[3]

The adjustment of concentrations is carried out starting from only one and even linear relation, obtained on an individual scale in one and same site, between bioaccumulated concentration of contaminant and the condition index. The formulate of adjustment retained is given by: **[ML] aj = [ML] mes + [a * (1/IC r - 1/IC mes)]** with [ML] = heavy metal concentration ; aj = adjusted; mes = measured ; IC mes = condition index measured ; IC r = reference condition index ; a = slope of the line.

The concentrations calculation of Cd and Zn adjusted, presented in figures 3 and 4, shows that the variations in time are reduced compared to the measured concentrations.

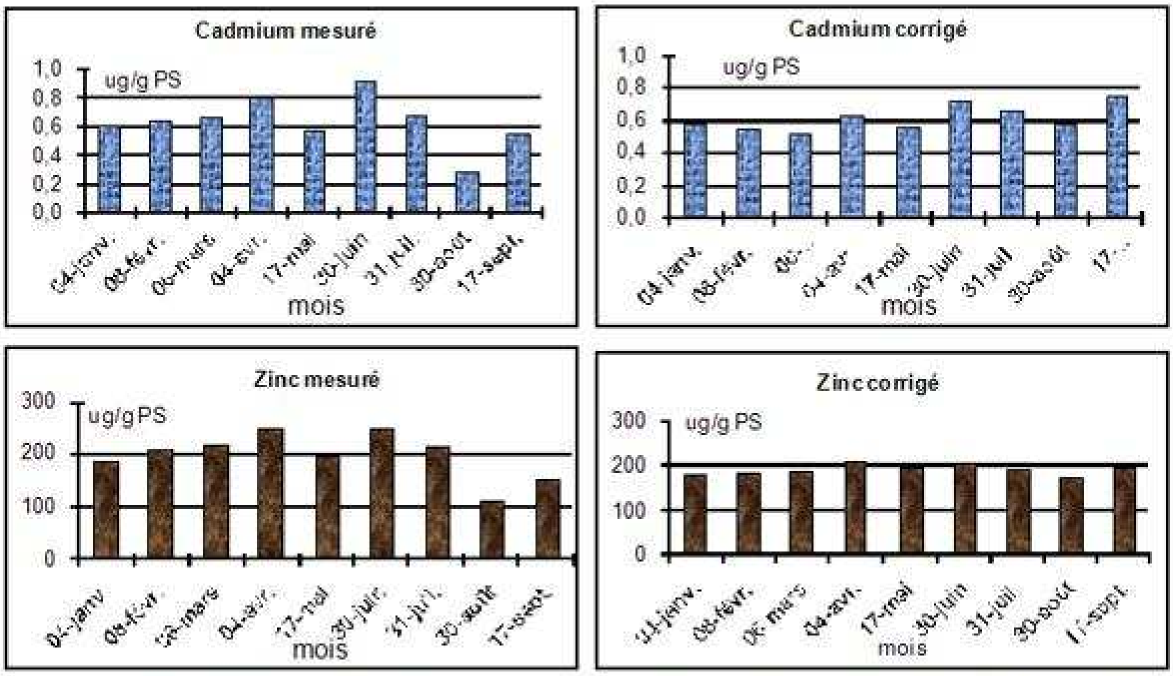


Figure 3 : Temporal evolution of the contents Cd and Zn measured and adjusted in the mussel at Dar bouazza.

[Fig3]

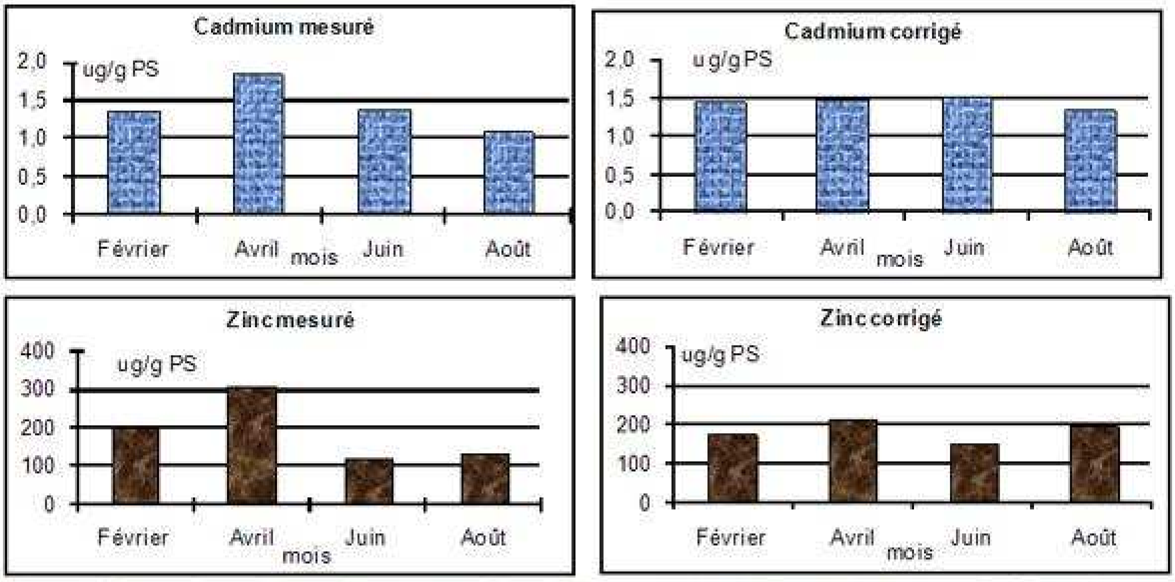


Figure 4 : Temporal evolution of the contents Cd and Zn measured and adjusted in the mussel at Ouled ghanem.

[Fig4]

Conclusion: It comes out from this work that : the metal contents in mussel (*Mytilus galloprovincialis*), in the marine environment, recorded, show temporal variations and agree with other research (Kaimoussi and al, 1998) which binds these variations to the metabolism and the reproduction cycle in mussel (gametogenesis and egg laying). The calculation of condition index made it possible to take into account the various factors related to the physiology of the species and to adjust the rough results. The temporal variations, recorded in the case of the zinc and cadmium, do not remain significant after taking into account of this index. Separately mercury, we recorded a significant correlation between heavy metal concentrations and reverse condition index. The accumulation adjustment of heavy metals in the mussel made it possible to compare the contamination levels of both located studied and to deduce that the Ouled ghanem is contaminated more by cadmium than Dar bouazza.
Lastly, the accumulation rates of Cd, Hg and Zn recorded in the mussel *Mytilus galloprovincialis* do not indicate any notable contamination of the sites having been subject of this study.

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A chemical substitution study for a wet processing textile mill

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In textile processing industry, large amount of water is used during dyeing and finishing processes. While Integrated Pollution Prevention and Control (IPPC) Reference Document on Best Available Techniques (BAT) for the Textiles Industry indicates that water consumption varies from 70 to 250 l/kg fabric depending on the techniques applied, many sources from South Africa indicated that the specific water intake for the textile industry varies from 95 to 400 l/kg fabric depending on the type of processes used and water efficiency. While, 20-230 m^3 of water is needed to produce 1 ton of textile fabric in Turkish factories. The total quantity of chemicals used in textile mills varies from 10% to over 100% of the weight of the cloth and the chemical loads are generated mainly due to the residues from preparation, dyeing, finishing, sizing, and other operations. Therefore, the amount of water discharged and the chemical load of textile effluents are the major environmental concern in the textile industry. Textile manufacturing generates solid, hazardous and air pollutant wastes, on the other hand, wastewater, by far, is the largest waste stream. For the textile industry, in general, the effluent is highly-colored, high in BOD and COD, has a high conductivity and is alkaline in nature. These factors combine to present numerous operational problems in municipal wastewater treatment works, which are biological processes and not intended for the breakdown of complex organic molecules. The presence of metals and other dye compounds inhibits microbial activity and in some cases may cause failure of biological treatment systems. Therefore, substitution of chemicals having lower hazard potential for chemicals having higher hazard potential should be a main focus for pollution prevention. Chemical (Material) Substitution is defined as “the replacement or reduction of hazardous substances in products and processes by less hazardous or non-hazardous substances, whilst achieving an equivalent functionality via technological or organizational measures”.

Many studies indicate that material or chemical substitution can bring pollution prevention and increase in cost effectiveness together. Admittedly, treatment costs can be reduced by introducing more biodegradable chemicals in production lines.

Literature indicates that pollution prevention by replacing sizing agents, surfactants, urea, solvent, acid, and reducing agents with environmentally friendly chemicals can be achieved.

In this study, a wet processing textile mill in Turkey was investigated. The average annual capacity of the mill is 20 thousands tons of ring yarn and 40 millions meters of denim fabric. In all wet processes, the ground water is used and the daily average water consumption of the mill is about 3500-5000 tons. The total monthly consumption of the chemicals is about 1000 tons and over 100 textile chemicals including dyestuffs and auxiliaries are used in the wet processes in the factory. The mill has its own wastewater treatment plant (WWTP). The daily wastewater generation of the mill depends on daily number of processes operated. All washing water, pretreatment, dyeing, softening and sizing solutions are discharged to the WWTP.

The outcomes of this study indicated that:

- There were totally 8 out of 128 chemicals identified as environmentally problematic. The total monthly consumption of these problematic chemicals is about 50 tons. This value represents the 5% of the total monthly consumption amount of all chemicals used in the factory.
- By substituting the dyestuff A with a lower sulphide product, reduction in amount of sulphide (due to the use of sulphur dyes) discharged to WWTP is achieved up to 76%. That is, this substitution decreases the inhibition risk on microorganisms in the WWTP and consequently prevents a possible fail in WWTP system due to use of sulphur dyestuffs.
- Substitution of Complexing Agent C* for C has led to improvement of the biodegradability of wastewater samples collected from the first post-washing tank just after the dye bath by at least 25% (from 38% to 64%). Also, by this substitution monthly 3100 kg of COD load to the WWTP is prevented.

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Fixing ammonium and phosphate during the anaerobic digestion of waste activated sludge

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The anaerobic treatment of waste activated sludge (WAS) in domestic wastewater treatment plants is a conventional technique to reduce the volume of the sludge as well as to stabilize the sludge prior to its use in land applications. This technique can be profitable if the production of biogas is controlled during the operation. The use of WAS has been recommended to improve the chemical and physical properties of soil (Debosz et al., 2002 and Wong and Su, 1997) and the plant growth (Wang et al., 2003). Indeed, WAS has been widely used in various forms for land application in many countries. According to the United States Environmental Protection Agency (1999) estimates, 41% of sewage sludge, produced in the USA, was applied to the land in 1998.

However, the long-term land application of sewage sludge and compost from waste materials may be limited by accumulation of harmful heavy metals and pathogens in soil (Stabnikova et al., 2005) and by contamination of the surface and subsurface waters with nutrients, especially with P (Celen et al., 2007). There are two major approaches to solve contamination problem of receiving environments by nutrients based on the removal of P using chemical or biological techniques versus the recovery of P by solubilisation of sludge using physical, mechanical, biological, or chemical methods. There may be, however, another approach aiming the removal of P during anaerobic digestion of sludge by fixing the major form of P, which is orthophosphate, in the form of insoluble PO43- containing species, such as, magnesium ammonium phosphate (MgNH4PO4.12H2O), which is commonly named as struvite.

Considering the composition of struvite, fixing PO43- ion helps also decreasing concentration of NH4+ in the system, which is another nutrient contaminating the water resources.

The release of large amounts of N and P during anaerobic digestion was reported by different researchers (Cheng and Liu, 2002; Martin et al., 2003; Noike et al., 2004; Demirer and Chen, 2005a) and can be attributed to the anaerobic bioconversion of proteins and solids. The low treatment efficiencies observed for total Kjeldahl N and total P are also expected since anaerobic digesters are known to reduce negligible amounts of nutrients (Lusk, 1998). As a result, the effluent from the anaerobic digesters needs to be further treated in the plant to prevent eutrophication (extraordinary growth of algae as a result of excess nutrients in water bodies) of rivers, lakes, and seas (Lau et al., 1997; Trepanier et al., 2002). There are many studies investigating the potential of struvite formation in the effluent of anaerobic digesters for the removal and recovery of nutrients (Burns et al., 2001; Nelson et al., 2003Schuiling and Andrade, 1999; Uludag-Demirer et al., 2005; Zdybiewska and Kula, 1991; Li et al., 1999; Altinbas 2002; Tunay et al., 1997; Battistoni et al., 2000; Munch and Barr, 2001), but few has attempted to promote the formation of struvite inside the digesters. There are only two studies reported in which struvite precipitation was carried out during the AD of a food waste and dairy manure in a digester by Lee et al. (2004) and Uludag-Demirer et al. (2007) respectively. Lee et al. (2004) reported that struvite precipitation obtained by the addition of Mg2+ during AD led to 67 % N and 73 % P removal. While Uludag-Demirer et al. (2007) stated that the anaerobic reactors supplemented with struvite precipitating chemicals removed considerable amounts of NH3 (10-23%) relative to the Control Reactor. They further noted that this is a clear indication of the possibility of using anaerobic digesters for forced struvite precipitation as well.

There are several advantages associated with the controlled struvite formation in an anaerobic digester, such as,

a) Controlling the formation of struvite in the anaerobic digester may stop struvite depositing problem in hydraulically significant points, pipeline and valves, of the reactor.

b) The removal of NH4+ and PO43- ions can be achieved in-situ as they are released during anaerobic digestion.

c) The quality of the anaerobically digested WAS can be improved by mixing it with a less soluble, slow release mineral like struvite (Salutsky et al., 1970; Beal et al., 1999; and Miles and Ellis, 2001).

d) The recovery of N and P to be used for land application is accomplished without a significant cost. Considering especially the limited resources of P containing rocks in the world, fixing P more within the sludge would help to compensate the need for P containing fertilizer for agricultural purposes. For example, Western European phosphate industry has set up a goal to reuse 25% of the recovered phosphate as raw material within 10 years (Fielding, 2000).

The released N and P during anaerobic digestion of WAS can be fixed within the anaerobic sludge by promoting the formation of struvite by adding limiting struvite-forming ions. Formation of struvite requires a molar concentration ratio of Mg:NH4:PO4 of 1:1:1 in the solution. Typically Mg2+ ion is one of the limiting reactants for struvite formation and its addition has become common practice to initiate struvite formation in wastewaters (Doyle and Parsons, 2002). Although NH4+ and PO43- concentrations are in excess compared to Mg2+, the concentration of PO43- may also control the formation of struvite since its molar concentration is generally lower than that of NH4+. Therefore, there may be also need to add PO43- ion to increase the amount of NH4+ fixed in the form of struvite.

The aim of this study was to fix both NH4+ and PO43- ions in the form of struvite as they are released during the anaerobic digestion of WAS by adding ions limiting struvite formation. The anaerobic digestion of WAS was carried out in batch reactors containing high initial concentrations of only Mg2+ or Mg2+ and PO43- ions. The performance of anaerobic digestion of the WAS as well as ammonia and phosphorus removals were tested under the conditions without pH adjustment.

The results obtained showed that the performance of the anaerobic digestion changed between -4.0 % and 15.4 % for Mg2+ concentration range of 277.17-529.27 mg/L and PO43- concentration of 377.4-2372.4 mg/L. There was an increase in the concentration of NH4+ in all reactors during digestion, but the extent of concentration increase was controlled by increasing initial concentrations of Mg2+ and PO43-. The maximum removal of NH4+ ion (33.7 %) was observed in the reactor dosed initially by Mg2+ and PO43- of 467.17 mg/L and 2123.3 mg/L respectively. The concentration of PO43- ion was lower than its initial concentration in all reactors indicating considerable fixation of PO43- as it was released, by struvite and other solid species formation. The decrease in the concentration of Mg2+ ion similar to PO43- was accepted as evidence for the formation of solid species composed of Mg2+ and PO43- in addition to struvite.

The major findings of the study clearly indicated that WAS can be one of the major resources for P used in agricultural activities by improving the conditions for struvite formation. However, carrying out further research with a focus on characterization of sludge mixed with struvite and investigation of its properties, especially N and P release rate is necessary in addition to improvement of the technique so that more N and P can fixed within the sludge.

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Comparison of two methods for sampling tar in the gas obtained from peat gasification in Latvia

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Aims: Biomass is one of the important primary and renewable energy sources. At present, the biomass share in the world's total primary energy consumption is about 12%. It is estimated that it will further increase in developed countries to nearly 15% by 2010.

Gasification of biomass produces a raw gas mixture composed of hydrogen, carbon monoxide, carbon dioxide, water, methane, and various light hydrocarbons. The producer gas also contains several undesirable constituents, including dust (ash and char), ammonia, alkali (mostly potassium), sulfur, chlorine, and tar. Tar is a complex mixture of aromatics including a certain fraction of polycyclic aromatic hydrocarbons. The tar derived from biomass gasification gets condensed if the temperature of the process equipment such as fuel lines, filters, engines and turbines is lower than the tar dew point, and then it blocks and fouls this equipment.

Some authors suggest that the tar derived from gasification can be generally classified into two types: a heavy tar which is difficult to gasify and is finally polymerized to produce char and a light tar which is easy to gasify and is finally converted to gas. A significantly larger amount of heavy tar results from coal gasification than from gasification of wood biomass.

According to literature, a high-temperature biomass tar mainly consists of highly stable aromatic compounds such as benzene (60-70%), naphthalene (10-20%), and other polyaromatic hydrocarbons (10-20%). So benzene is a tar component that prevails in the gas obtained from real biomass gasification.

Many authors though claim that benzene as a real biomass gasification gas component does not present any problem as its combustion is clean and results in no clogging, so a complete removal of benzene is not required. Therefore, benzene should be treated as a separate compound and excluded from the definition of tar. Although many authors excluded benzene from the definition of tar, it seems that more attention should be paid to the analysis of benzene in producer gases because, first, benzene plays a certain role in the formation of ozone, which causes a variety of respiratory effects. Second, benzene is also an air toxic ranked tenth in the list of hazardous air pollutants and recognized as having both acute and chronic effects on human beings, including reproductive and developmental effects.

Methods: In the present work, a cold solvent-trapping (CST) and solid-phase adsorption (SPA) methods for determining concentration of tar compounds have been chosen for comparison. Traditional methods are based on cold solvent-trapping. They proved very impractical for light compounds and require lengthy sampling times (15-60 minutes per sample). By contrast, the SPA method is easy to handle, and one sample is typically taken in only 1 minute. The SPA method was developed by The Royal Institute of Technology in Sweden [1] to measure tar compounds ranging from benzene to coronene. According to this method, tars are sampled by collecting on a column with a small amount of an amino-phase sorbent. But this method does not allow for determining such volatile organic compounds as benzene, toluene, and xylenes. In the previous papers [2,3], an improved system for sampling tar, namely, equipped with one more adsorbent cartridge loaded with another sorbent, was suggested and described. The best results were obtained while using activated coconut charcoal as the second sorbent. So, a modified sampling device consisting of 500 mg of amino-phase sorbent and 100 mg of activated coconut charcoal was chosen as optimal for sampling tar and volatile organic compounds it comprises in synthesis gas produced in biomass gasification.

For research in real life context, the Circulating Fluidised Bed (CFB) gasifier situated in eastern Latvia (Rēzekne region) was chosen. Peat extracted several kilometres from the gasifier was employed as biomass.

When a cold solvent-trapping method is used, the producer gas flows through a series of impingers containing 2-propanol, whereas in a solid-phase adsorption method it passes through two adsorbent cartridges loaded with 500 mg of aminopropyl-bonded silica adsorbent, packed loose (the surface area is about 400 $m^2 g^{-1}$ - 600 $m^2 g^{-1}$, the size of a particle 50 µm, an average pore size 60 Å, from Alltech) and 100 mg of activated coconut charcoal (the surface area 1070 $m^2 g^{-1}$, the size of a particle 20/40 mesh, from SUPELCO). A gas chromatograph with the MS system Shimadzu GCMS-QP2010

(Shimadzu Corporation, KYOTO, Japan) was used for the analysis. All 2-propanol from all impingers was mixed and analysed by the gas chromatograph. The tar from amino-phase sorbent was eluted with dichloromethane and analysed. The activated coconut charcoal was placed in a vial where carbon disulfide was added; then the solvent was analysed by GCMS. The amounts of tar in dichloromethane and in carbon disulfide were summed up as the total amount of tar on two adsorbents analysed by the solid-phase adsorption method.

Results: Adsorption efficiency of the tar compounds on sorbents and in solvent depending on the amount of gas drawn through was explored. For this, various volumes of synthesis gas was drawn through the both adsorbent cartridges at the flow rate 100 mL min^{-1} or through the impingers train at the flow rate 5 L min^{-1}. The gas was drawn for various periods of time, namely 1, 2, and 3 minutes for solid-phase adsorption method or 15, 30, and 45 minutes for cold solvent-trapping method, so 100 mL, 200 mL, and 300 mL or 75 L, 150 L, and 225 L of gas were drawn through adsorbents and solvent respectively. Adsorption efficiency of the tar compounds on sorbents and in solvent depending on synthesis gas temperature was studied. For this, definite volumes of gas was drawn through the both adsorbent cartridges at the flow rate 100 mL min^{-1} for 1 minute or through the impingers train at the flow rate 5 L min^{-1} for 15 minute. The sampling line was heated till 200°C, 250°C, and 300°C.

Conclusions: The method suggested by Brage at all. is special in that it might be difficult to determine such a volatile organic compound as benzene whose concentration in synthesis gas is high, and the sampling volume exceeds the breakthrough volume of the adsorbent. The present research showed that benzene does not get completely adsorbed on the first adsorbent, the amino phase one. Furthermore, effectiveness of adsorption depends on a range of factors, amount of the gas drawn through the adsorbent, gas temperature, concentration of tar and benzene in synthesis gas being among them. But under practically all these conditions benzene that was not adsorbed on the first sorbent gets fully adsorbed on the second one, i.e. activated coconut charcoal. The sum total of the benzene adsorbed on both sorbents allows for the assessment of the entire amount of benzene in synthesis gas. More than 60 compounds sampled with cold solvent-trapping method and 50 compounds sampled with solid-phase adsorption method were identified by comparing the spectra obtained by manual interpretation with the spectra from the NIST mass spectral library. SPA is a more accurate method than those using impingers, especially for determining such volatile organic compounds as benzene, toluene, and xylenes, due to using a second sorbent, activated coconut charcoal. By contrast, the CST method proves more accurate for determining heavy tar, due to a much bigger volume of the sampled producer gas.

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Chemical and ecotoxicological characterization of solid residues producing during the co-pyrolysis of plastics and pine biomass

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Aims: The aim of this work was to perform the integrated chemical and ecotoxicological characterization of leachates from pyrolysis solid residues. Since this carbonaceous residue produced in the pyrolysis process is not considered economically interesting, is usually disposed of in landfills. Therefore, it is important to evaluate the stability of these residues, namely to evaluate the possible leaching of toxic compounds, such as inorganic and organic contaminants, and the ecotoxicity of the eluates. The determination of organic contaminants in leachates was focused on the volatile group because these are also the compounds with lower molecular weight and higher water solubility showing a high environmental mobility. The inorganic characterization was performed in the wastes and their leachates with the determination of the following heavy metals: Cd, Cr, Cu, Zn, Pb and Ni. The toxicity of a waste or of its leachate can also be evaluated by using ecotoxicological tests wherein the purpose is to characterize the toxicity level of the sample regardless of its chemical composition. In this study, the ecotoxicity of the leachates was determined with the bio-indicator Vibrio fischeri.

Methods: Pyrolysis: A mixture of 70% (w/w) pine biomass and 30% (w/w) plastics (mixture of PP, PE and PS) was subject to pyrolysis in a 1L autoclave, during 15 min at a temperature of 400ºC with an initial pressure of 40 MPa.

At the end of the essay, the reaction products were: 15% (w/w) gases, 30% (w/w) solids and 45% (w/w) liquids. One part of the solid fraction was submitted to a Soxhlet extraction with dichloromethane (DCM). The DCM extracted residue (Residue A) and the non-extracted solid residue (Residue B) were submitted to the following analyses.

Determination of the organic matter content in residues A and B: The organic matter content was determined by proximate analysis by measuring the weight loss associated with the combustion of the solid samples in a microwave muffle furnace. The samples were heated from room temperature (25±1ºC) until 550±1ºC, with increments of 50ºC.

Determination of the content in heavy metals in residues A and B: The samples were submitted to a microwave acid digestion (HCl:HNO3, 3:1, v/v) in closed PTFE vessels. A selected group of heavy metals were quantified in the digested samples using atomic absorption spectrometry (AAS): chromium, cadmium, copper, zinc, nickel, and lead.

Leaching tests: The leaching methodology followed the standard leaching test ISO/TS 21268 - 2 [35]. The waste is mixed with the leaching solution in a single-stage batch test performed at an L/S ratio of 10 L/kg, at a constant temperature of 20±2ºC. The containers (capped glass bottles) were shaken in a roller-rotating device at 10 rpm, for a period of 24±0.5 h. The leachants used were the following: a calcium chloride solution with a concentration of 0.001 mol/L (according to the standard ISO/TS 21268 - 2 [35]) and a DCM solution of 0.2% (v/v).

Table 1 shows the codes used for the different leachates obtained.

Leachate code	Type of leachate
1	Residue A (previously extracted with DCM) leached with CaCl2 0.001M solution
2	Residue B (not extracted) leached with CaCl2 0.001M solution
3	Residue A (previously extracted with DCM) leached with DCM 0.2% (v/v) solution
4	Residue B (not extracted) leached with DCM 0.2% (v/v) solution

Determination of BTEX and other volatile benzene derivatives in leachates: The concentration of BTEX compounds in the leachates was evaluated by headspace static sampling and gas chromatography with flame ionization detection (GC-FID). Other volatile benzene derivatives present in the leachates were identified by headspace sampling and gas chromatography hyphenated with mass spectrometry. Identification was performed by comparison of the retention times and mass spectra of the compounds with standards. Some organic contaminants were tentatively identified by the comparison of their mass spectra with references from the Wiley and NIST spectra libraries.

Determination of inorganic parameters in leachates: The heavy metals Cd, Cr, Cu, Ni, Pb and Zn were quantified in the eluates using atomic absorption spectrometry.

Ecotoxicological characterization of leachates: The ecotoxicological parameter analyzed in the leachates was the luminescence inhibition of the bacterium Vibrio fischeri ("Azur Environmental Microtox® system").

Results: Weight loss by combustion: Residues A and B lost more than 99% of their initial weight, when heated up to 450ºC, which indicates that they were mainly composed of organic matter. The initial weight loss, in the temperature range of 25ºC to 200ºC was negligible for residue A and higher than 30%(w/w) for residue B. At temperatures between 200ºC and 450ºC both residues lost weight at a similar rate until stabilizing at a steady value of less than 1% of their initial weight. Both residues presented a stable residual weight between 450ºC and 550ºC. The extraction with DCM was, therefore, an efficient process for the removal of the volatile organic fraction from these residues, and thereby reducing their toxic load but not affecting the semivolatile and non-volatile fractions.

Contents of some heavy metals in the solid residues: Both residues showed significant amounts of the metals Cr, Ni and Zn, while the concentrations of metals Cd, Cu and Pb were below the respective detection limits. Cr, Ni and Zn are also the metals that were detected in higher content in the pine biomass and were subjected to a magnification factor in the solid residues due to the pyrolysis process.

Chemical analysis of the leachates: All the metals analyzed were present in those concentrations below the detection limits, except for Ni and Zn, which also appeared in significant quantities in the solid residues. Ni and Zn were easily released from the residues to the eluates.

The solid residues also had high concentrations of Cr (especially residue A) but this metal was not detected in the leachates. This suggests that the chromium detected in the residues should be Cr(III), which has low solubility at neutral pH values No BTEX compounds (or other volatile organic compounds) were detected in leachates 1 and 3 obtained from residue A, which confirms that the extraction of the residue with DCM was an efficient method for the removal of volatile organic contaminants. Leachates 2 and 4 showed a contamination with toluene, ethylbenzene and o-xylene. Besides for the confirmation of the presence of BTEX, several other aromatic hydrocarbons (PAHs, furans and other benzene derivatives) and other organic compounds such as phenolics were also detected. The compounds detected in higher relative concentration, besides BTEX, were cumene and propylbenzene, which are two volatile aromatic hydrocarbons.

Ecotoxicity Tests: Leachates 2 and 4 have shown the highest toxicity. Concentrations of 0.53% and 1.27% (v/v), has induced a 50% of luminescence inhibition to V. fischeri, probably due to the presence of higher contents of organic compounds, as residue B was not previously extracted with DCM, as it happened with residue A from which were obtained eluates 1 and 3. Therefore, no significant toxicity was detected in leachates 1 and 3. Only for an exposure period of 30 min was it observed that there was a 50% luminescence inhibition for concentrations of approx. 70% (v/v).

Conclusions: The extraction of pyrolysis solid residues with an appropriate organic solvent is an efficient method for the reduction of their content in organic contaminants and, therefore, a reduction on their ecotoxicity. The solvent used in this work, DCM, showed a good capacity for the removal of volatile organic compounds but the weight loss experiments showed that the residue extracted with DCM still contained a reasonable amount of non-volatile organic matter. Residue A was not classified as ecotoxic by the biological criteria but is considered ecotoxic by the chemical criteria. The toxicity of residue A is associated with the presence of the heavy metal Ni. Residue B was classified as ecotoxic by both chemical and biological criteria and its toxicity was attributed to the presence of organic contaminants. Residue B was also classified as a non-inert residue according the 2003/33/CE Council Decision.

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An investigation of sludge gasification in a fixed bed reactor

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In the history of Turkey, tanning has been the one of the most important art. Today, tanning is a centralized sector and they are usually placed at organized industrial district like Tuzla, Çorlu, Menemen, Gerede, Torbalı, and Denizli. Large volumes of water are used in the leather industry and concentrated wastewater and solid waste are produced during the leather production.

Wastewater problems are solved by using modern wastewater treatment plants. However, sludge is produced during the wastewater treatment and handling and disposal of this sludge is another problem. In Turkey, about 600 tons/day leather is processed and it is estimated that over 100.000 tons/year of sludge is produced from this sector. Environmental effects of sludge are being one of the most important problems in our country like all over the world. As a result, the most suitable disposal method and reuse alternatives of sludge are very important topics for researchers.

Gasification and pyrolysis are similar processes; both decompose organic waste by exposing it to high temperatures. Both processes limit the amount of oxygen present during decomposition; gasification allows a small amount of oxygen, pyrolysis allows none. In other words, gasification and pyrolysis limit or prevent oxidation.

In the scope of this study, the gasification of sludge produced in wastewater treatment plant of a leather industry, which is classified as hazardous waste, was investigated in order to obtain energy from produced syngas. At the same time, waste minimization was also obtained. The sludge was characterized and gasification process was applied by using a laboratory scale model plant. Advantages and disadvantaged were discussed.

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Biological and oxidative treatment of textile dye bath effluents by brown coal packed reactors

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This study is an investigation of treatment options for highly polluted azo reactive dye baths generated from cotton textile dyeing processes. Reactive dyes are considered to be the most problematic group for treatment. Among these azo reactive dyes are commonly used in textile sector. For this group, 40 to 90% of dye is fixed to the fabric during dyeing, whereas, the rest appears in wastewater causing high COD, and color which requires advanced treatment. The dye-bath residue is resistant to microbial degradation due to the high amount of recalcitrant organic compounds. Segregation of dye baths having strong pollutant characteristics from less strong reusable streams and treating them separately facilitates recycling of relatively clean streams in the process.

To emphasize the advantages of water segregation and reuse by in-plant measures, the treatability characteristics of dye-baths by oxidative and biological methods were investigated in this study. For this purpose, 60oC remazol dye bath effluents, from two different textile plants, with COD concentrations of 900-1250 mg/l , were used. The enterprises are classified under the knit fabric dyeing finishing subcategory, involving production of cotton, polyester and polyamide products.

Dye bath treatability study was conducted by setting-up and operating fixed bed and upflow-fluidized bed type reactors. Brown coal char (lignite), as a low price material, was used as a media for adsorption and for biofilm formation in the reactors. The reactors were fed various dye-bath concentrations throughout the study. The overall hydraulic retention time was around 1.3 days. Acclimatization of the attached growth type bacteria to the dye baths used was practiced during the early stage of the study. To investigate the effect of the adsorption/biological oxidation and chemical oxidation processes on biodegradability, experiments were carried out on the inlet and outlet of reactors. Chemical oxidation was achieved by ozonation. Breakthrough conditions and criteria were determined for the packing media. Biodegradable and inert COD fractions of remazol dye-bath were assessed by BOD-28 and oxygen uptake rate (OUR) measurements. Color was measured by absorbance measurements at three different wavelengths.

80-90% COD and 93-99.9% color removal efficiencies were achieved for the treatment of strong remazol reactive dye baths by using reactors with brown coal. The high efficiency obtained was due to the biofilm layer and the high adsorption capacity of brown coal.

An approach, based on respirometric measurements, was practiced to determine inert COD fractions (CODi). The study revealed that 50% of total COD was initially inert, which indicates the dye baths investigated consisted considerable amount of non-biodegradable fraction. CODi for the 30-minute ozonated dye-baths was measured to be about 40%. Although, ozone oxidation was not significantly effective on COD removal, CODi fraction was reduced by 10% which means 10% increase in biodegradability was achieved by ozonation.

Furthermore, the packed reactor was proved to be efficient in removing CODi by adsorption. It was shown that about 65% of the CODi initially present in the dye bath was removed by the brown coal bed and about 40% reduction in CODi was achieved by 30 minute ozonation.

Key words: textile wastes, azo reactive dye bathes, physicochemical treatment, biofilm, adsorption, biological treatment, BOD28, COD fractionation, ozonation

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Gasification of yarn industry wastes in a fixed bed reactor for syngas production

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Diversity of vital requirements and of the wastes produced at the end of continuously developing production processes increase day by day. Storage, incineration and recycling methods which are used to dispose domestic wastes produced in huge amounts can not be applied to industrial wastes due to their complex structure and amounts.

It is of great importance to prevent production of/to dispose industrial wastes within the scope of quality standards commonly applied in industries and environmental awareness. Production wastes are classified into groups; liquid wastes are sent to waste water treatment facilities for treatment purposes and solid domestic wastes are disposed through recycling, storage or incineration. On the other hand, special disposal methods are applied for the industrial wastes classified as “hazardous waste” and generally thermal processes are preferred. Of thermal process; each of incineration, pyrolysis and gasification processes enables not only disposal of the waste in such way to prevent any environmental damage buy also utilization of the waste as an alternative energy source to be used in power generation.

It is widely known that, in recent years, pyrolysis and gasification methods have been used to generate power from particularly the biomass of agricultural activities. Pyrolysis process enables thermal decomposition of organic matters and wastes under oxygen-free conditions. Gasification, on the other hand, is a syngas gas production process under controlled-oxygen and high temperature conditions (with minimum liquid and solid products). Different gasification processes are used to generate power from biomass. It is possible to produce H2 or H2+CO gas, to be used in power generation, from biomass via pyrolysis and gasification processes, which are two thermo-chemical transformation methods.

This study aimed at determining the optimum conditions for gasification of wastes produced during polyester yarn production process in a fixed bed gasification reactor of laboratory size and analyzing the production of H2 or H2+CO gas for power generation. Gasification process was carried out at 700-10000C temperature interval, under atmospheric pressure and at different air ratios (0.15-0.45). Components at gas phase -CO, CO2, H2 and CH4- were measured during each trial. The results of the trials have shown that appropriate gas products are produced through air gasification at high temperatures. Syngas production increases in parallel with the reactor temperature. The increase in the amount of the air fed into the medium prevents tar production on one hand but decreases the amount of the syngas as well as the calorific value of the produced gas on the other.

Key words: Yarn; Polyester; Air; Gasification; Fixed Bed; Syngas

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Determination of volatile aromatic compounds in leachates from pyrolysis solid residues using a HS-GC-MS method

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Aims: One of the industrial processes that produce wastes with an important organic content is pyrolysis. In this process, the wastes are subjected to high temperatures in the absence of oxygen, producing a hydrocarbon mixture (vapour and liquid fractions) and a carbonaceous residue (the solid fraction). The solid fraction is not normally reused and is, therefore, disposed off in landfills. Therefore, it is important to evaluate the stability of these residues, namely to evaluate the possible leaching of toxic compounds. The carbonaceous residue is saturated with the components of the liquid fraction so it must be extracted with an appropriated organic solvent to reduce its organic load before it can be discarded.

In this work we validated an analytical method for the quantitative determination of 15 volatile benzene derivatives (benzene, toluene, ethylbenzene, xylenes, cumene, propylbenzene, 4-ethyltoluene, tert-butylbenzene, 1,2,4-trimethylbenzene, 1-methylpropylbenzene, butylbenzene, 1,4-diethylbenzene and 1,2,4,5-tetramethylbenzene) in leachates from pyrolysis solid residues, using headspace gas chromatography with mass spectrometry detector. The solid phase microextraction technique was also performed and detection limits were calculated. The non volatile organic contaminants present in these leachates were screened with an innovative method (dispersive liquid-liquid microextraction) and a tentative identification of these contaminants was made.
Methods: Pyrolysis and leaching: a mixture of pine biomass and plastics (mixture of polypropylene, polyethylene, and polystyrene) was subjected to pyrolysis, and part of the solid residue produced was subjected to extraction with dichloromethane (DCM). The extracted residue (residue A) and the raw residue (residue B) were submitted to the leaching test ISO/TS 21268-2. Two types of leachates were obtained: leachates from residue A (leachate 1) and leachates from residue B (leachate 2).
Sampling and extraction procedures: the static HS was performed using the following optimized experimental conditions: equilibration time, 30 min; extraction temperature, 60ºC; vial volume, 50 ml; sample solution volume, 25 ml; injection volume, 0.5 ml. The HS-SPME was performed using two types of fibers, namely, the PDMS 100µm fiber and the Carboxen-PDMS 85µm fiber. The experimental conditions for the SPME experiments were: vial volume, 50ml; sample volume, 25 ml; equilibration time, 30 min; extraction temperature, room temperature; extraction time, 5 min; desorption time, 3 min; desorption temperature: PDMS, 200ºC and Car-PDMS, 250ºC. The experimental conditions for the DLLME technique were: sample volume, 5ml; extraction solvent volume, 50µl carbon tetrachloride (CCl4); disperser solvent volume, 0,5ml acetone, injection volume, 1µl.
GC-MS analysis: the chromatographic analysis conditions for the static HS experiments were performed using a Focus gas chromatograph equipped with a split-splitless injector, a TR-V1 capillary column (30 m × 0.25 mm I.D. × 1.4 µm film) and a Polaris Q mass spectrometer detector. The carrier gas was helium at 1.5 ml/min; samples were injected at 60ºC in the splitless mode with a splitless time of 1 min and a split flow of 50 ml/min; the interface and ion source were kept at 220ºC; the oven temperature program was as follows: 32ºC (hold 5 min), up to 100ºC (5ºC/min), up to 250ºC (10ºC/min) and finally up to 280ºC (50ºC/min, hold 1min). The MS system was operated in the full scan mode with a mass range from 50 to 200 amu. For the HS-SPME experiments the equipment and GC-MS conditions were the same except for the injection temperature (250ºC for the Car-PDMS fiber and 200ºC for the PDMS fiber).
The GC-MS analysis of the DLLME extracts was performed in the same equipment but with a DB5 column (30m x 0,32mm x 1µm); the injector was kept at 220ºC; the carrier gas flow was 1 ml/min and the injection volume was 1 µl. The oven temperature program was: 35ºC (hold 3 min) and heating ramp up to 300ºC (5ºC/min). The MS detector was operating with the full scan mode covering a mass range from m/z 50 to 650. All other chromatographic parameters were the same as in the previous experiments.
Results: Determination of benzene derivatives using different extraction techniques: the efficiency of static HS, HS-SPME and DLLME as techniques for sampling the 15 volatile benzene derivatives was tested. The limits of detection and of quantification for each analyte were evaluated as, respectively, 3,3 and 10 times the signal-to-noise ratio of the corresponding chromatographic peaks. Most of the analytes tested (except benzene and toluene) were detected in the pg/L range using static HS or HS-SPME. The DLLME technique showed higher LODs than the other techniques but nevertheless allowed the detection of most analytes in the low ng/L range. All of the techniques tested are adequate to the detection and quantification of these 15 aromatic hydrocarbons in leachates from industrial residues at levels that are considerably lower than the limits proposed in legislation for the hydrocarbons of the BTEX group (100 µg/L).
The static HS was chosen for method validation because it is a solvent-free technique and considerably less expensive than SPME. Moreover, the HS-SPME showed some fiber memory effects that required periodic conditioning of the SPME fibers.
Validation of the HS-GC-MS method for the determination of benzene derivatives in leachates: benzene was the analyte with better analytical performance, with the larger linearity range, higher correlation coefficient and lower LOD. The repeatability of this proposed HS-GC-MS method was evaluated by analyzing five replicates at two different concentration levels, a low level of 500 ng/L and a high level of 1000 ng/L. The relative standard deviation (R.S.D.) for the lower level of concentration was between 8.4 and 13.5%, and for the higher level of concentration the R.S.D ranged from 4.5 to 14.5%. Following the validation of the developed method, duplicates of the leachates samples were analyzed after spiking them with the analytes standard solution to a final concentration of 500 ng/L. Good recoveries were achieved in both leachates, although benzene showed the lower recovery.
Analysis of leachates using the HS-GC-MS method: the validated HS-GC-MS method was used to

determine the concentrations of the 15 benzene derivatives compounds in both leachates. None of the 15 benzene derivatives compounds were detected in leachate 1 obtained from residue A that was previously extracted with DCM which means that this extraction step was efficient in removing the majority of the volatile organic fraction from pyrolysis residue. Leachate 2 showed a high contamination with toluene, ethylbenzene, o-xylene and cumene in concentrations from 9 to 511 ppb, with predominance of toluene. Almost the 15 organic compounds were detected in this leachate, except tert-butylbenzene and 1,2,4,5-tetramethylbenzene. Besides the benzene derivatives selected, other intense peaks were detected that correspond to phenolics, PAHs, other benzene derivatives, among others.

DLLME-GC-MS analysis: DLLME technique showed higher LODs than HS sampling technique, however, the sensitivity of this technique (in the ng/L range) with volatiles is remarkable.

The DLLME method was also used to extract and identified less volatile compounds present in the leachates. Again, was confirmed the presence of a great variety and high amounts of phenolic compounds.

Conclusions: the HS-GC-MS method developed allowed to achieve very lower LODs, comparable with those achieved with HS-SPME. Comparing with other sampling techniques HS has the follow advantages: does not use organic solvents, avoids the introduction of non-volatiles into the inlet of the gas chromatograph, which is not desirable, and does not require expensive or complex instrumentation.

DLLME is a technique that presents a powerful preconcentration method using small quantities of solvents. This technique shows it self as applicable to extract volatiles with relatively low LODs.

P 58

Sorption of uranium (VI) ions from aqueous solution by nanoscale magnesium oxide

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Nanoscale metal oxide particles, almost by definition, possess high surface areas, many pores, coordinative unsaturated surface cations and anions. Metal oxide nanoparticles are highly active for a large number of reactions that are important in both pollution control and chemical synthesis. Magnesium oxide (MgO, periclase) is used in a variety of applications such as refractory materials, pharmaceutical, waste remediation, glass industry and catalysis. MgO has a simple NaCl structure and it can be prepared with widely ranging surface areas. MgO exhibits remarkable reactivity and rates of adsorption which is strongly dependent on the crystallite size, surface area, structure defects and acid-basic site concentrations. Magnesium oxide was chosen for this study as it is one of the cheapest and most easily prepared adsorbents amongst metal oxide nanoparticles.

The objective of this work was to study the sorption of uranium (VI) ions on magnesium oxide from aqueous solutions. The adsorption of uranium (VI) from aqueous solutions onto magnesium oxide nanoparticles have been prepared via hydroxide precipitation from aqueous solutions, followed by the thermal decomposition of the hydroxide. Magnesium oxide was characterized by X-ray powder diffraction, scanning electron microscope (SEM) and BET specific surface area (SSA). The relative importance of test parameters like concentration of adsorbate, pH, contact time and temperature on adsorption performance of magnesium oxide for U (VI) ion were studied. Typical adsorption isotherms (Langmuir, Freundlich, Dubinin-Raduskevich) were determined for the mechanism of sorption process. Also the thermodynamic constants (ΔH°, ΔS° and ΔG°) were calculated.

P 59

Isokinetic emission sampling of PCDD/Fand PCB and inhibitory effect of (NH4)2SO4 during combustion of wood and hospital waste in a small scale furnace

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Incineration is an important method for the treatment and decontamination of biomedical and health-care waste. This process of high-temperature (850ºC to 1100ºC) dry oxidation may successfully reduce organic and combustible waste to inorganic, incombustible matter and results in a very significant reduction of waste volume and weight. Furthermore, incineration is certainly capable to destroy the bacteria and viruses and the material of attached pathogens: the paper, plastic, glass and metal. Consequently, acidic gases are generated from the chlorinated organic plastics present; toxic metals are liberated from the pigments and the additives of the paper and plastic products as well as discarded thermometers; and dioxins and furans are formed from the chlorine in the waste 1. Unfortunately, incineration is not a clean process 2. In 2001, emissions from hospital waste incinerators in the European Union were estimated in 200-400g I-TEQ year-1, accounting for 25% of the total polychlorinated dibenzo-p-dioxins/polychlorinated dibenzofurans (PCDD/F) emissions 3. Currently, two major options of primary and secondary measure are known to comply with the limit value of 0.1 ng/m3 TEQ PCDD/F, which was demanded by the European directive 2000/76/EC. Compared to the primary measures, the installation and operation costs of secondary measures such as air pollution control systems are much more expensive and cannot be considered for small hospital waste facilities. Primary measures include adjustment of operation conditions (temperature, turbulence, air flow, residence time) and focus on mechanism of inhibition of selective compounds or inhibitors, which can inhibit the formation of PCDD/F substantially 4. In the recent years the usage in a laboratory scale experiment of some sulfur and nitrogen compounds such as hydroxylamine-O-sulfonic acid (H2NSO4H); amidosulfonic acid (H2NSO3H); sulfamide (H2NSO2NH2); ammoniumthiosulfat ((NH4)2S2O3); urea+sulfur ((NH2)2CO+S) (1:1); ammoniumsulfat ((NH4)2SO4) become beneficial into prevention of PCDD/F and PCB emissions 5. Especially successful concerning the abatement of organic toxic pollutants in flue gas was the addition of only 5% (NH4)2SO4 to the coal/PVC-containing solid waste fuel 6. The objectives of this work were to investigate and determine the PCDD/F and PCB emissions during the co-combustion of wood and hospital waste, operated in a pilot scale plant, and to study the effect of (NH4)2SO4 as potential inhibitor on PCDD/F and PCB emissions.

The pilot application was performed on an existing domestic grid-stove in a small-technical scale. A combustion scheme to generate PCDD/F and PCB was designed and constructed. The system consisted of two chambers. A small domestic stove was installed a room where the emission sampling was executed.

Wood, hospital waste and (NH4)2SO4 with different proportions were used for combustion to produce or prevent exhaust gas containing PCDD/F and PCB. Five combustion experiments of 100% wood, case (a); 75% wood / 25% hospital waste, case (b); 75% wood / 25% hospital waste, case (c); 71% wood / 24% hospital waste / 5% (NH4)2SO4, case (d) and 71% wood / 24% hospital waste / 5% (NH4)2SO4, case (e) were performed. The prepared fuel mixtures were combusted in batches. The basic fuel in all cases was eco-farm wood briquettes (BIO-Brennstoff-Vertrieb-GmbH). Wood combustion is known to be an important source of PAH and is also considered to be responsible for a significant percentage of the PCDD/F emissions. Chlorine addition to the fuel mixtures (b), (c), (d) and (e) was in the form of PVC-containing hospital materials such as hoses and blood bags (Sarstedt AG & Co). Further various non-chlorine containing hospital materials were also included in the fuels such as syringes, needles, swabs (all from NeoLab Migge Laborbedarf-Vertriebs GmbH), gloves and sticking plaster (Söhngen GmbH). The pooling of the hospital waste was estimated so that the PVC-containing materials prevail two times the non-chlorine containing ones. Additionally, in case (d) and (e) the inhibitory effect of (NH4)2SO4 was examined inhibit the emissions of PCDD/F and PCB. The amount of the tested N-and S-containing compound was adjusted based on our previous studies to correspond to 5% of the total fuel mixture enforcing successful inhibition results 6.

Emissions of PCDD/F and PCB were sampled isokinetically by employing an automatic sampling system MRU 4000 (GSM, Neuss, Germany). The sampling was performed according to DIN EN 1948-1 and the cooled probe method was applied. The volumetric contents of the gas components were measured on-line with a gas analyser TESTO 350 (Lenzkirch, Germany).

The soot particles were filtered by a glass cartridge filled with glass wool. The gaseous PCDD/F and PCB were absorbed by using a cartridge filled with Supelpak 2 (cleaned XAD resin from Supelco).

Prior sampling the cartridge was spiked with 13C-labeled sampling standards. After each sampling the water of the condensate flask was poured over the XAD resin and then discarded. The probe and all glassware were rinsed first with n-hexane and afterwards with toluene. The rinsing solutions were kept and used for Soxhlet extraction later.

The sample preparation, instrumental analysis, and quantitation were performed according to DIN EN 1948 part 2 and 3. The XAD resin was extracted in a Soxhlet apparatus with toluene for 24 h. Prior extraction 13C-labeled standards were spiked onto the cartridge. The clean-up of the extracts and instrumental analysis were performed. The instrumental analyses were performed by HRGC/HRMS.

Low PCDD/F and PCB concentrations are were measured during wood combustion (case (a), Table 1). Due to higher chlorine contents of the hospital waste the dioxins emissions are sufficiently increased during the next two experiments of wood and hospital waste, case (b) and (c). However, in case (d) and (e) where $(NH_4)_2SO_4$ was involved, the reduction of the PCDD/F and PCB concentrations were determined to an average of approximately 50%. Continued experiments of wood/hospital waste fuel without inhibitor and with inhibitor, case (c) and (e) effect the highest and lowest toxic emissions, respectively. Consequently, the results in case (e) compared with case (c) manifest a minimization of 66% for both PCDD/F and PCB emissions.

	a	b	c	d	e
PCDD/F pg (WHO-TEQ)/Nm3	8167	107931	189536	85458	65711
PCB pg (WHO-TEQ)/Nm3	192	1810	3691	1366	1260

Furthermore, furans prevailed over dioxins in all cases with a ratio ranging between 2.5 and 4.5,indicating a relatively constant behavior of PCDD/F formation.

Isokinetic sampling of PCDD/F and PCB during combustion of wood and hospital waste was performed in a pilot plant. The influence on toxic emissions of 5% $(NH_4)_2SO_4$ in toxic fuel was further investigated. As a result the lowest PCDD/F and PCB emissions were observed during the combustion of fuel blends containing wood only. In contrast to case (a) the forward four campaigns described with presence of chlorine in the fuel mixture lead to significant toxic emissions.

Consequenstly, the experiments of wood and hospital waste, case (b) and (c) the higher percentage PVC content results in the higher toxicity. In agreement another study suggested that even 3% PVC in the fuel is able to result increase in PCDD/F concentrations. Several recent publications reported also correlations between chlorine input and the emission of polychlorinated aromatics based on de novo formation of the PCDD/F. The contradictory effect was found for the experiments (d) and (e). Despite presenceof PVC content the detected toxic emissions enhance to be lower. The relationship manifests the strong inhibition effect of $(NH_4)_2SO_4$ by continued combustion of wood and hospital waste such as case (e).

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P 89

Selfassembling ionic liquids - not really a „green“ alternative!

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Ionic liquids, usually classified as salts molten below 100℃, have gained more and more attention over the last years. Their physical and chemical properties can be tuned task-specific by the individual choice of cation and anion. Furthermore, their outstanding qualities like: high conductivity, non-flammability, high thermal stability, outstanding catalytic properties and negligible vapour-pressure, have made them an interesting, and so-called “green”, alternative to organic solvents, which led to first industrial applications on ton-scale. In addition to the classical, well-established short-chain ionic liquids, the selfassembling long-chain imidazolium salts arouse scientific interest. Despite this intense interest in the use of ionic liquids only little attention has been paid to their environmental risk. Only a few studies have been made considering the biodegradability and the toxicity of classical short-chain ionic liquids - two crucial factors considering their bio compatibility. No investigations focusing the long-chain derivatives are known. In this study the cytotoxicity of long-chain 1-methyl-3-alkylimidazolium chlorides on the human cancer cell line HeLa has been examined, showing disproportionately high cytotoxic impact compared to short-chain ionic liquids and common cationic surfactants.

P 90

Organochlorine pesticides in human hair from the Madeira River Basin - Amazon

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Aims: Human hair, as a potentially additional tool for the public health surveillance, is providing a non-invasive sampling with additional merits, e.g. stable matrix, easy for collection, short and long-term exposure tracings and so forth. The aim of this study is to determine the concentrations of organochlorine pesticides, especially dichlorodiphenyltrichloroethane (DDT) and two of their metabolites, in human hair collected in the Madeira river Basin.
Methods: Hair samples were collected from riverines communities of Madeira river, Amazon-Brazil. These communities have a historic use of DDT to control the Malaria vector - Anopheles sp. All participants were volunteers, who agreed to the test and only the occipital part of hair was sampled. The samples were analyzed by gas chromatography/mass spectrometry for the above compound, including its metabolites: dichlorodiphenyldichloro ethylene (DDE) and dichlorodiphenyldichloroethane (DDD) and other pesticides such as heptachlor and hexachloorcyclohexane (HCH).
Results: A total of 63 samples were included in the study. The DDTs profile consisted in 4,4’-DDT and 4,4’-DDE which levels correspond to 93.6% of sum DDT. The levels of DDT isomers were between 0.70 and 5,394ng/g, of DDE isomers 0.14 and 3,177ng/g and of DDD isomers 0.61-108 ng/g. To heptachlor: 84.1% of the samples presented some concentration between 0.07 and 7.82ng/g. For the isomers of hexachlorocyclohexane the percentages was: α-HCH 0.43% (0.02-0.40ng/g), β-HCH 96.4% (0.03-2.69ng/g) and γ-HCH 3.12%(0.02-12.19ng/g).
Conclusions: The results suggest recent exposure to fresh DDT and support the possibility of using hair as a suitable indicator for the assessment of long-term exposure organochlorine pesticides.

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Extraction of tannins from bioorganic waste as a pretreatment of biogas production

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The hypothesis underlying this project is that tannins are toxic to microorganisms, which produce methane from carbohydrates contained in banana waste; and that removing tannins prior to fermentation will enhance the yields of methane production. In addition to toxicity problems, the formation of complexes between tannins and proteins or minerals decreases the bioavailability of these nutrients to the microorganisms of interest.

The project aims at developing methods suited for the pre-treatment of banana peels, before the latter are processed for biogas production. The expected benefits of such pre-treatment are: i. the decrease of the tannin content in banana waste with the subsequent increase of methane production yields, ii. the simultaneous production of tannin extract, which constitutes raw material for possible application in green chemistry (e.g. synthesis of new drugs from vegetal material). In order to ensure the success of the project, the studies are driven mainly by consideration of two constraints, i.e. the compatibility of the pre-treatment with the biogas production process situated downstream and the economical sustainability of such additional process step. In order to attain these objectives, the strategy implemented during this project addresses the three following questions:

1. Is there any method suited for the extraction of tannins from banana peels?
2. Does resulting bio-waste still meet the requirements for subsequent production of biogas?
3. Does this method constitute an economically sustainable solution and can the tannin extract be used for further applications in life science industry?

Poster Session 3 - Ethics, Policy, Education, Risk & Reach and Green Processes

P 42

Electrophilic bromination of alkenes: Environmental, health and safety aspects of new alternative methods

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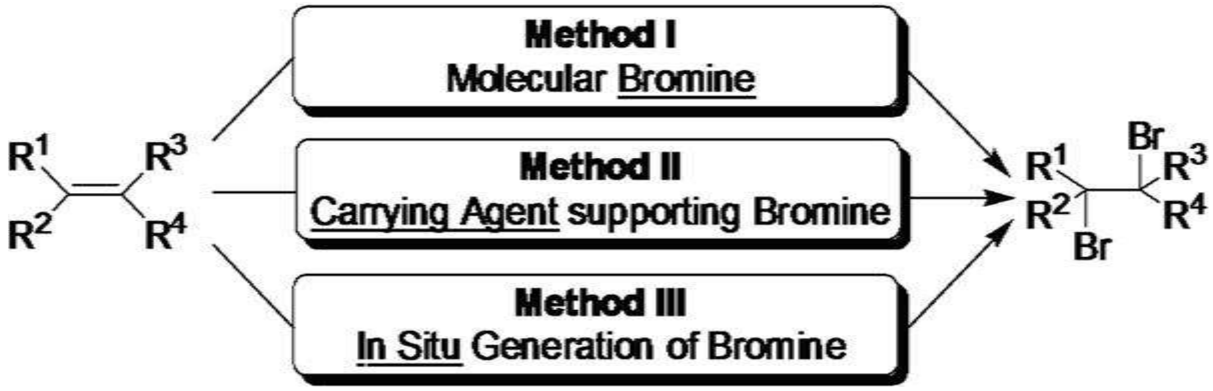
Bromination of organic molecules is an important task in synthetic organic chemistry. Bromine can be added to alkenes and alkynes yielding vicinal dibromides; they are mostly obtained stereospecifically as trans-dibromides. [2]

Until now bromination was done mostly by use of molecular bromine as reagent mainly in chlorinated solvents. But during the last ten years several new bromination methods have been developed, which circumvent bromine as reagent, mostly to avoid the hazard potential of this reagent. We evaluate different synthetic methods (Figure 1).

- Bromine in a traditional manner: Elemental bromine is added.
- Carrying system: Bromine is supported onto an auxiliary material.
- In situ generation of bromine: Using oxidization agents bromide ions are converted into bromine in situ.



[Figure 1]
Figure 1 Considered Methods for electrophilic bromination of alkenes

However, in alternative protocols other problematic substances are used. In addition, resource demands often seem to be significantly higher than in the traditional manner. Different chemicals used in these methods are subjected to a qualitative discussion concerning flammability, toxicity, eco-toxicity etc.. [1] Quantitative considerations[3] regarding resource consumption and waste production are performed[1] on the basis of mass balances.

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P 60

Adsorption of diuron in three moroccan soils: Influence of two sewage sludge

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In Morocco, the production of sewage sludge resulting from domestic water treatments increases; a way of recycling this sewage sludge is to apply them such as manure or fertilizer of the soil, because of their high contents in organic matter (OM) and in mineral nutriments. The objective of this study was to assess the influence of application of sewage sludge on the adsorption of diuron on three Moroccan soils (Dehs, Tirs and Mnasra) choosing for their physico-chemicals properties. The sewage sludges used in this study were collected from the wastewater treatment plant of Agadir: a fresh sewage sludge floating on the water surface (FSS) and dried sewage sludge decanted (DSS). The adsorption of diuron was measured using a batch equilibrium method. The isotherms of adsorption of diuron were well adjusted by the equation of Freundlich (r2 > 0,98). For the three unamended soils, the affinity of diuron was the following one: Dehs > Tirs > Mnasra, with respective Kf of 9.0, 4.0 and 2.0; these results are positively related with the organic carbon (OC) contents of the three soils (1.51%, 1.27% and 0.52%). Diuron shows more affinity for organic matter from the Dehs soil than for the Tirs and Mnasra soils. For both sewage sludges, the Kf adsorption coefficients of the amended soils were greater (from 5.1 to 14.6) than those of the unamended soils (from 2.0 to 9.0). For the three soils, the adsorption of diuron was not influenced by the nature of sewage sludge in spite of their different OC contents (118 g.kg-1 for the DSS and 201 g.kg-1 for the FSS). In conclusion, the sewage sludge addition sampled from wastewater treatment increased the diuron adsorption for the three studied soils, and thus could reduce the risk of contamination of groundwater by herbicides.

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Availability of heavy metals and As in oxidized and reduced dredged sediments

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Introduction: The industrial development and emission of mainly anthropogenic chemicals led to contamination of aquatic sediments with heavy metals and organic micro-pollutants. Sediments contaminated with heavy metals do not posses any significant risk, when kept under the water level as their solubilisation is minimal at anaerobic conditions. However, they can be exposed to oxygen when the water level drops after an incidental inundation, like in the case of rivers: Elbe (Ger) in 2002, Odra (PL) in 1997 and Rhine (Ger, NL) in 1995, leading to an increase of redox potential and affecting the (bio)availability of heavy metals. Moreover, sediments are dredged worldwide from the bottom of the rivers, channels and harbours both for environmental and navigation purposes [5]. Nowadays about $40x10^6$ m^3 per year of sediments are dredged in the Netherlands [10]. Temporary storage of polluted dredged sediments can be regarded as a low cost remediation technique [6]. However, oxygenation in a temporary disposal site is desirable for the aerobic degradation of organic pollutants (e.g. PAHs and mineral oil), but it may lead to mobilization of trace metals. Therefore, there is a need for characterization of sediments in dredging or sediments disposal projects [1], in which the changing metal availability by periodical redox changes is studied. Our study was focused on the effect of redox potential changes with respect to mobility of Zn, Cr, Pb, Cu and As in dredged sediments.

Material and methods: Five dredged sediments originated from three disposal sites in the Netherlands were studied. A simple extraction procedure with a neutral unbuffered salt solution (0.01 M $CaCl_2$) was used to investigate the behavior of available fractions of metals and As in batch tests conducted for 21 days under oxic and anoxic conditions [3]. Aerobic experiments were performed in closed batches (1000 ml) with a sufficient large headspace $O_2/N_2$21/79 (v/v %) to avoid anoxic conditions. Anaerobic experiments were performed in 120 ml bottles with N_2 /CO_2 80/20 (v/v %) by a gas change device (five gas volume changes). The total metal contents in sediments were determined by microwave-assisted *aqua regia* (HCl/HNO_3, 3:1) according to [7] and analysed by Inductively Coupled Plasma Mass Spectrometry (ICP - MS, Elan 6000, Perkin-Elmer, detection limit 0.1 μg/l - 2000 μg/l). The concentrations of metals in the liquid phase of supernatants were determined by centrifugation (15000 rpm over 10 min), and analysed by ICP - MS. The following ions: Cl^-, SO_4^{2-}, NO_2^- , NO_3^-, were analyzed using Ion Chromatograph (DIONEX DX 600). The total sulfur concentration was determined using Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) (Spectra-Flame, Spectro - detection limit 0.1 mg/l -100 μg/l). The gas composition of O_2, CO_2, and N_2 was measured by Gas Chromatograph TCD MFC 800, Fisons. The nitrogen compounds: NH_4^+, total NO_2^- and NO_3^- and PO_4^{3-} were determined using Segmented Flow Analysis (SFA).

Results and discussion: All sediments had the: neutral pH (pH 6.8-7.6), reduced state (Eh 55-146 mV), and a wide range of dry weight (24.1-74.9%) and organic matter (6.2-27.6%) contents. The highest initial metal contents in sediments (dry weight) were of: 1294 mg Zn/kg, 1014 mg Cr/kg, 781 mg Pb/kg, 206 mg Cu/kg and 486 mg As/kg, thus exceeded both: Polish and Dutch intervention values for heavy metals in soils [2,4]. The mass balances were performed to follow the use of oxygen and the production of CO_2, NO_3^-, SO_4^{2-} and PO_4^3. In all sediments the CO_2 production was rather low and the production of NO_3^- and PO_4^{3-} was minimal. The total oxygen consumption is represented in Figure 1 (e.g. for sediment II), as well as the products in which oxygen is present. Data show that not all O2 that was consumed, was found in O_2 containing products (see sum of products in Figure 1). This is most probably due to binding of O_2 to carbon in the solid matrix [9].

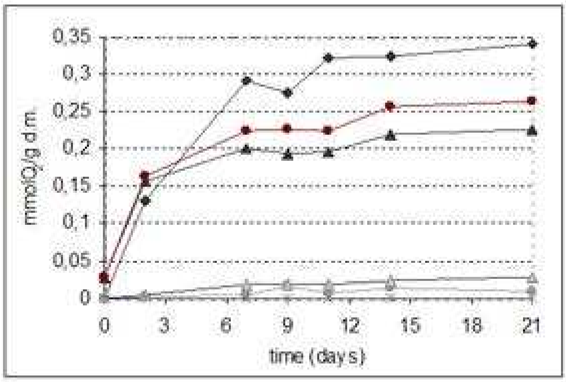

Figure 1. The oxygen consumption (diamonds) is presented with time as well as the products in which oxygen is present as CO_2 (grey circles), PO_4^{3-}(star) NO_3^- (grey triangle), and SO_4^{2-} (black triangles). The sum of oxygen in moles is presented by the black circles.

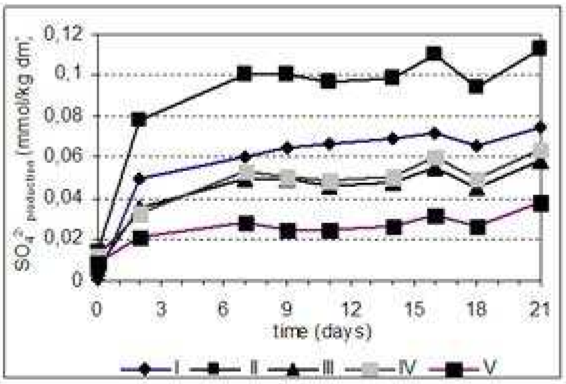

Figure 2. SO_4^{2-} production in sediments under aerobic conditions.

[1]
Sulfates showed to be the major products of the O_2 consumption in all studied sediments (Figure 2). An increase of the redox potential had a strong effect (about 40-80%) on the oxidation of reduced sulfur compounds to SO_4^{2-}). The increase of redox potentials resulted in an increased solubility of heavy metals in all sediments (e.g. for sediment I in Figure 3). The highest Zn concentrations were of 33070µg/l (after 12 days) and of 377µg/l for Cr (after 2 days), thus are far above the maximum allowable Polish and Dutch standards for ground- and surface-waters. Similar results were obtained for other sediments. Zoumis et al. [11] showed that oxidation resulted in mobilization of some heavy metals. Zn was significantly higher released in all sediments due to its weaker binding properties. Pb and Cu are generally less mobile elements in the environment [8].

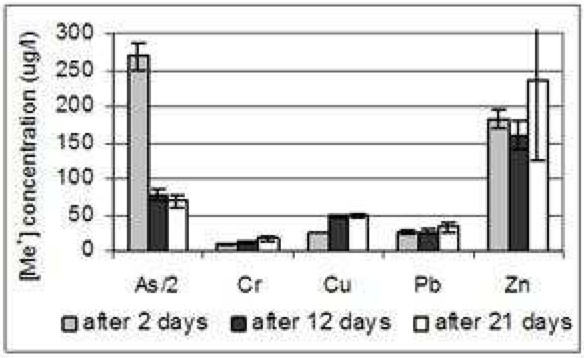

Figure 3. Solubility of heavy metals under aerobic conditions after 2,12 and 21 days.

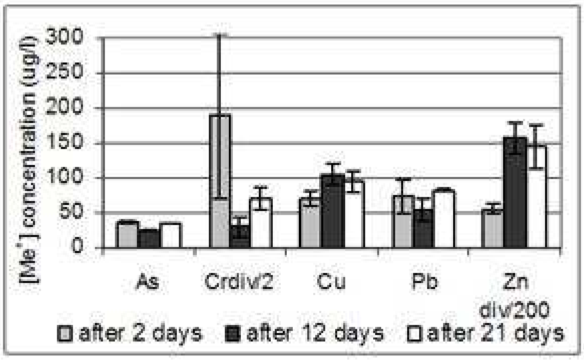

Figure 4. Solubility of heavy metals under anaerobic conditions after 2,12 and 21 days.

[2]
To avoid scaling problems in the Figure 3 the concentrations for Cr and Zn were 2 respectively 200 times higher than in Figure 3. In a control at anaerobic conditions, the concentrations of heavy metals were minimal in comparison to aerobic conditions. However, an increase of As concentration in liquid phase up to 134,5 µg/l (after 2days) was observed in sediments highly contaminated with As (e.g. for sediment III in Figure 4), exceeding the maximum allowable Polish and Dutch standards. Studies at low Eh also confirmed the release of As from anaerobic lake sediments [9]. Concentrations of As were 2 times higher than presented in Figure 4 to avoid scaling problems.

Conclusions: Changes of redox status play an important role in metals availability in sediments. Although the increase of redox potential led to a solubilization of about 1,4% of Zn and less then 1% of Cr, Pb and Cu of their initial concentrations in sediments, still the metal concentrations in the liquid phase exceeded standard values for surface water quality. The mobilization of heavy metals was less than 2%, however, too high from an ecotoxicological point of view. As such redox changes may occur within days, thus attention should be paid to sediments that are transferred to floodplains after flooding events. Oxidation of sediments should be avoided as far as heavy metals release is concerned.

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P 62

Hazard from polluted sediments in Poland and possible ex-situ methods of their clean-up

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Introduction: Sediments contaminated with inorganic and organic micro-pollutants are major environmental problem in many Western and Central European countries. Data taken from SedNet indicate that 100-200•10^6 m^3 are produced yearly in Europe [3]. In the 1990s, in several rivers and lakes high accumulation of heavy metals and persistent organic pollutants (POPs) was observed in Poland. Since than, sediments have been regularly sampled to control level of concentration as well as their changes in time. The extensive investigations of these compounds are carried out in the frame of State Programme of Environmental Monitoring in Poland [1]. The aim of the investigation was to establish the potential risk to aquatic environment from polluted sediments. Thus, the overview was made concerning the presence of some inorganic compounds as well as some POPs in sediments of Polish rivers and lakes. Heavy metals, polycyclic aromatic hydrocarbons (PAHs), chlorinated pesticides and polychlorinated biphenyls (PCBs) were studied. In the paper a brief overview of available ex-situ remediation options is also given [6]. Heavy metals and POPs concentrations in river and lakes sediments Poland is divided into seven Regional Water Management (RWM) zones of Gdansk, Gliwice, Krakow, Poznan, Szczecin, Warszawa, and Wroclaw [1].

TABLE1. Maximum heavy metals contents in sediments.

	As	Ba	Cd	Cu	Cr	Co	Hg	Ni	Pb	Sr	V	Zn
	mg/kg											
	Regional Water Management Gdansk rivers and lakes											
R	6	127	<0.5	78	72	5	1.360	**76**	54	68	18	198
L	14	159	1.6	22	29	**9**	0.187	19	79	276	**51**	168
	Regional Water Management Gliwice rivers											
R	60	**1003**	**30.7**	198	83	**16**	**6.420**	44	**2198**	136	32	**6600**
	Regional Water Management Krakow rivers											
R	8	221	26.2	56	47	12	**0.798**	43	399	113	45	1848
	Regional Water Management Poznan rivers and lakes											
R	14	317	6.6	66	69	13	0.687	30	96	88	33	693
L	15	157	1.9	26	23	6	0.245	17	102	457	31	319
	Regional Water Management Szczecin rivers and lakes											
R	<5	90	<0.5	19	9	2	0.167	5	22	31	9	185
L	7	109	1.4	45	24	7	0.415	16	104	582	37	**345**
	Regional Water Management Warszawa rivers and lakes											
R	14	240	14.2	111	84	12	1.830	31	165	**494**	27	1205
L	11	**184**	2.1	22	**31**	**9**	0.235	**21**	89	**641**	42	**225**
	Regional Water Management Wroclaw rivers and lakes											
R	**63**	249	4.4	**264**	**100**	**16**	2.010	52	104	54	**65**	437
L	**16**	171	**3.3**	**83**	25	8	0.454	17	**189**	278	40	315

R-rivers, L-lakes, the highest concentrations of each heavy metal id bolded

[1]
It was found that most of river sediments are polluted with heavy metals with the highest concentration of 63 mg As/kg, 1003 mg Ba/kg, 100 mg Cr/kg, 264 mg Cu/kg, 2198 mg Pb/kg and 6600 mg Zn/kg in sediments of the Odra River that is most industrial part of Poland (Table 1).

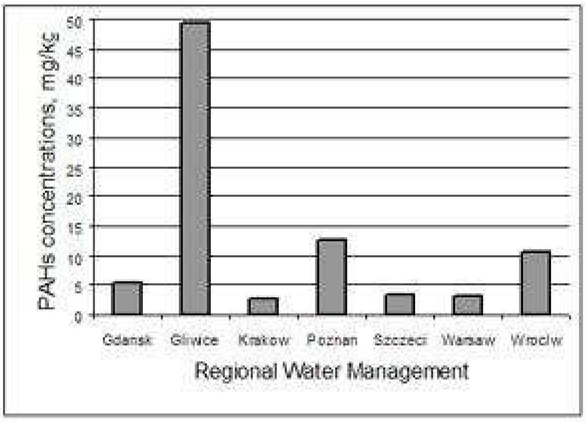


Figure1. PAHs contents in river sediments.

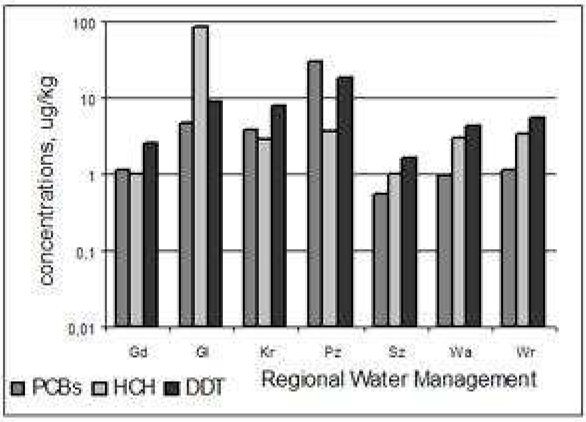


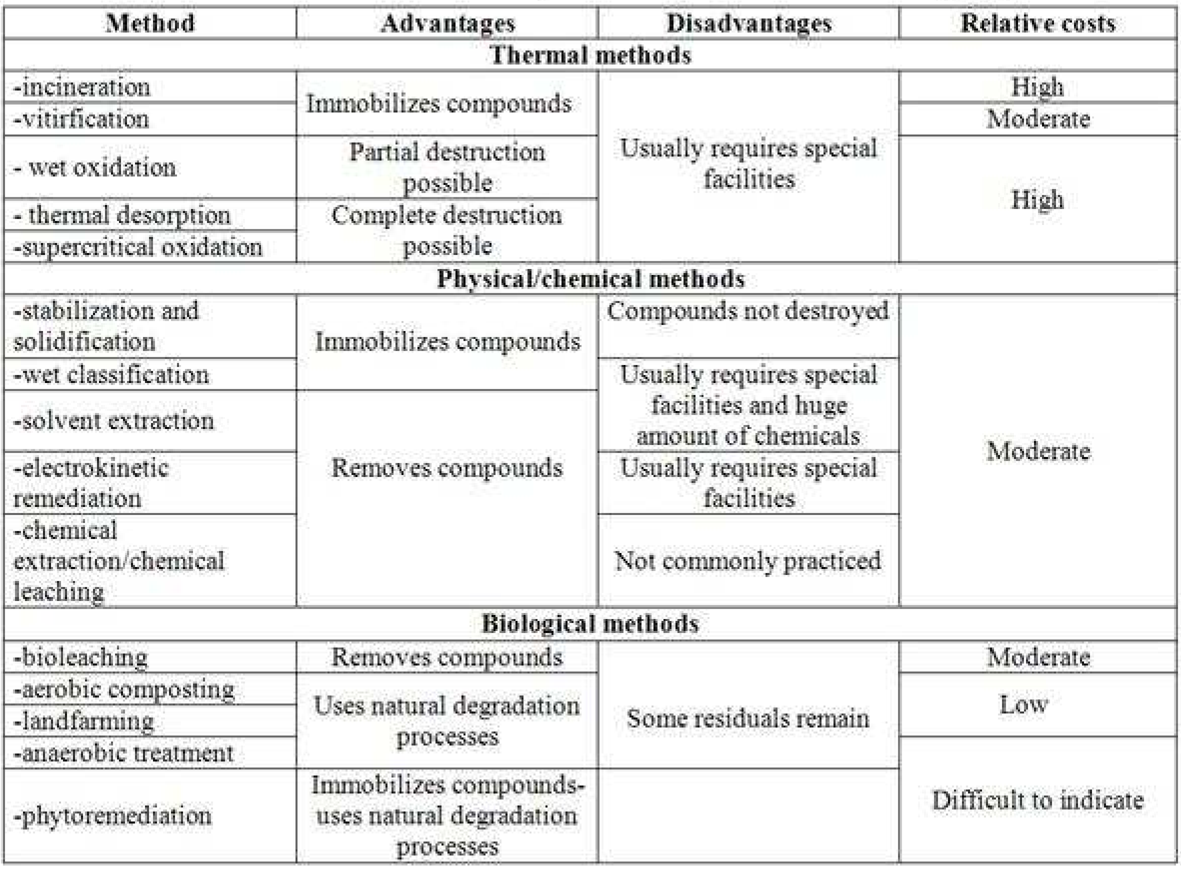
Figure 2. PCBs, HCH, DDT in river sediments.

[2]
Odra river sediments were also heavily contaminated with PAHs with the concentration of 49,406 mg/kg-sum of 15 PAHs listed by EPA (Figure1). Neighbourhood of urban-industrial centers resulted in the high concentrations of pesticides mainly found in river sediments. The most frequently detected pesticides were isomer HCH (Lindane) with the maximal contents of 83,250 µg/kg in Tyniec river (Figure 2). The presence of DDT and its metabolites was detected in almost all analyzed sediments with the maximal concentration of 18 µg/kg in Przemsza and Vistula River. They were also found in Warta river, the largest tributary of Odra river. The sediments of the Warta river contained also congeners of PCBs with the maximal concentration of 29,800 µg/kg (Figure 2).
Generally lower concentrations were found in lakes as compared to river sediments. It is due to the fact that the most of Polish lakes are located in the northern that is much less industrialized part of Poland. Maximal concentration of heavy metals was found in lakes in the north of Poland (in the neighborhood of Gdansk). The concentrations observed in sediments were of: 16 mg As/kg in Czarnowek lake, 184 mg Ba/kg in Slesinskie lake, 31 mg Cr/kg in Szelment Mały lake, 83 mg Cu/kg in Zaborsko lake, 189 mg Pb/kg in Czarnówek lake and 345 mg Zn/kg in Juno lake. The highest PAHs

concentration of the total 15 PAHs of 10,573 mg/kg was found in Białe Włocławskie lake. Concentrations of PCBs were minor below 1 μg/kg with the exception of two lakes- Białe Włocławskie and Tarnowskie Duze where PCBs exceeded 1 μg/kg. In the abovementioned lakes only one p,p -DDE metabolite occurred in the concentration varying from 0,5 to 5,6 μg/kg, whereas concentrations of HCH were minor with the exception of lake Biale Wlodawskie.

Ex-situ remediation methods: In ex-situ methods contaminated sediment is removed usually by excavation, and thereafter the sediment is treated on-site, or it is transported to another location and then treated. The overview together with various assessments of the remediation options is given in Table 2.

TABLE2. Ex-situ techniques used in sediments remediation [4,5,6]



Method	Advantages	Disadvantages	Relative costs
Thermal methods			
-incineration	Immobilizes compounds	Usually requires special facilities	High
-vitirfication			Moderate
- wet oxidation	Partial destruction possible		High
- thermal desorption	Complete destruction possible		
-supercritical oxidation			
Physical/chemical methods			
-stabilization and solidification	Immobilizes compounds	Compounds not destroyed	Moderate
-wet classification		Usually requires special facilities and huge amount of chemicals	
-solvent extraction	Removes compounds		
-electrokinetic remediation		Usually requires special facilities	
-chemical extraction/chemical leaching		Not commonly practiced	
Biological methods			
-bioleaching	Removes compounds	Some residuals remain	Moderate
-aerobic composting	Uses natural degradation processes		Low
-landfarming			
-anaerobic treatment			Difficult to indicate
-phytoremediation	Immobilizes compounds- uses natural degradation processes		

[3]

Conclusions: Following the concentration of inorganic and organic micro-pollutants in sediments since 1990 it becomes clear that sediments did not change significantly in quality [4]. Therefore, there is a need to find the possible methods of clean-up polluted sediments. In Poland there is a legislation concerning heavy metals and some POPs concentration in dredged sediments that are excavated from the bottom of the rivers, channels and harbors mainly for navigation purposes [2]. On the other hand there is scare information of studies on dredged sediments in Poland so far. However, in the near future contaminated sediments will most probably have to be dredged. The reason is that polluted sediments are a secondary source of pollution by inclusion of micro-pollutants formerly immobilized in sediments again into the environment cycle. As a consequence sediments may pose a threat for water organisms. None of presented ex-situ remediation options is a panacea for sediments remediation and often more than of these may be necessary to optimize the clean-up effort. Sediments are complex matrix composing of multiple pollutants and it makes most remediation effort costly and arduous.

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P 63

Oil-production by microalgae: an interspecific comparison

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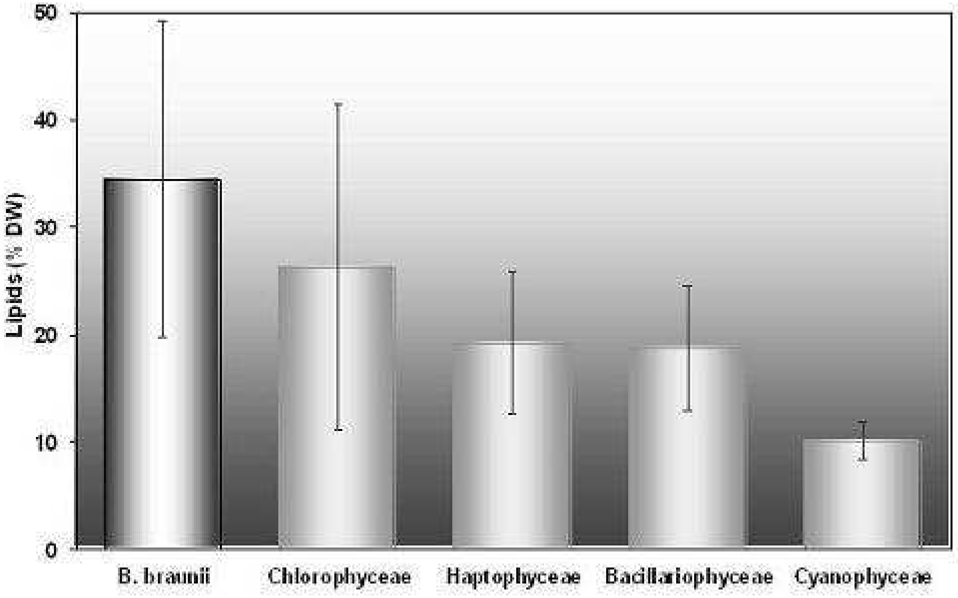
Introduction: Biodiesel derived from oil crops, as a renewable carbon-neutral alternative to petroleum fuels, is suitable for environmental sustainability. While conventional oil-crops, and even the best producers like rapeseed, castor been or oil palm, cannot realistically satisfy the existing demand for transport fuels, microalgae, as the most efficient primary producers of biomass, appear to be the only source of renewable biodiesel that is capable of meeting the global demand for transport fuels (Chisti, 2007). However, more research efforts are still needed to maximize the overall productivity and further enhance the cost effectiveness of the biofuel from microalgae strategy. In particular, small scale studies and research on the basic biological issues are recommended (Sheehan et al.,1998; Li et al., 2008).

Botryococcus braunii, which is a microalgal species characterized by a high capability to synthesize large amounts of biolipids, has been selected to investigate the oil productivity in most of the studies undertaken through the 1980s and heightened in the last 15 years (Metzger and Largeau, 2005). These studies were mainly focusing on the specific capacity of microalgae to accumulate cellular oil rather than to produce cellular biomass.

Aimed to find a balance between oil content and growth rate, such as to optimize the oil yielding of algae mass-culture systems, an interspecific comparison was performed on a large number of microalgal species belonging to different taxonomic classes.

Methods: More than 70 microalgal species/strains were considered to compare their potential of oil production. An extensive literature was analyzed to obtain a homogeneous data set of species-specific lipid content, as preferably measured in exponential-growing conditions, and expressed as percent of biomass dry weight. The literature review covered more than 25 years studies on microalgal physiology, starting from Shifrin and Chisholm (1981) to Dayananda et al. (2007) and enabled to characterize a large number of species, mainly belonging to the taxonomic classes of Chlorophyceae, Haptophyceae, Bacillariophyceae and Cyanophyceae. For most of the species the exponential growth-rate (doubling/day) data were coupled to lipid content to evaluate the rate of oil production in active-growth conditions

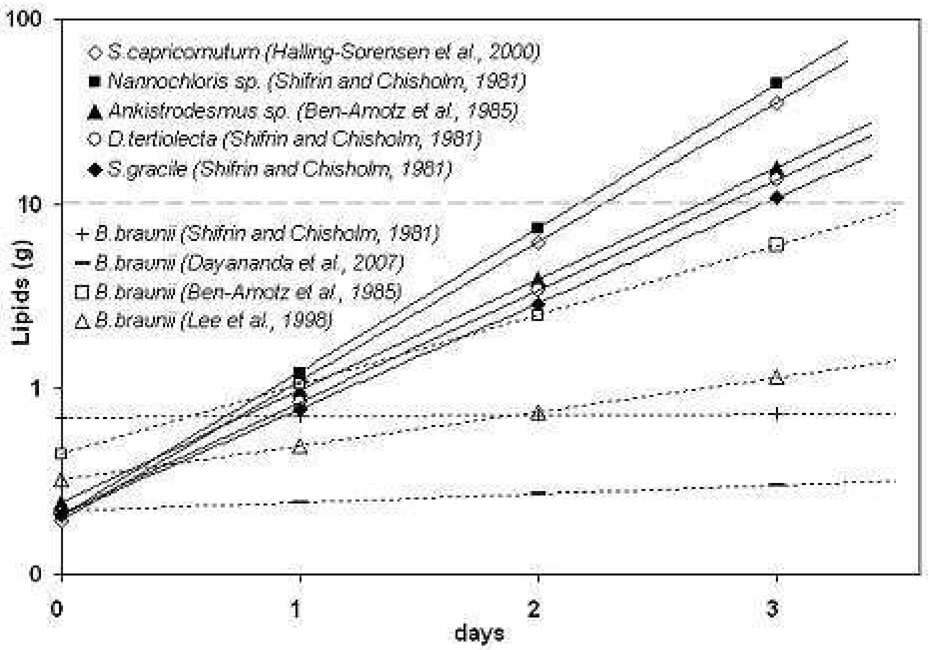
Results and discussion: A high variability of the lipid content (generally ranging from 10 to 70% of biomass) was found not only when comparing all the different microalgal species, but even within the same *B. braunii* species. In Figure 1 the mean lipid value with standard deviation, calculated for each taxonomic class, is compared with *B. braunii* value, calculated on 18 literature data measured on 6 different strains.



[Fig.1 Mean lipid content ± SD]

Comparing the algal classes, the Figure shows that the lipid content of Chlorophyceae (calculated on 26 species/strains) is the most similar to *B. braunii*. Haptophyceae and Bacillariophyceae exhibit mean values lower than 20%, while Cyanophyceae as low as 10%.

The rate of exponential-growth, coupled to lipid content, was used to simulate the specific trend of oil-production in microalgal mass-cultures, where exponential cell-growth is expected. Five species belonging to the same class of Chlorophyceae were selected and compared with *B. braunii* in Figure 2.



[Fig.2 Lipid production trend]

The lipid-accumulation trend, based on the fact that lipids double following the biomass-doubling, was calculated over 3 days, starting from one gram of biomass, for the freshwater species *Nannochloris* sp., *Selenastrum capricornutum*, *Selenastrum gracile*, *Ankistrodesmus* sp., and for the brackish *Dunaliella tertiolecta*. All the five selected species are representative for the class of Chlorophyceae, all being characterized by a mean oil content between 20 and 25% of biomass and by a doubling/day typically higher than *B. braunii*. For *B. braunii* four different literature-data sources, giving markedly different values both of lipid content (from 22 up to 70%) and of doubling/day (0.02 to 1.25), were used. As unexpected, after 3 days no one *B. braunii* reaches a lipid yield as high as 10 grams, while lipids accumulated by all the other five species exceed that limit, reaching up to 45 grams, following the growth rate as high as 2.6 doubling/day. As clearly suggested by the oil-production trends plotted in Figure 2, a specific lipid content as high as 70% cannot be effective unless coupled to high productivity-rates.

Conclusions: In spite of its capability to accumulate high levels of lipids in the cells, the use of *B. braunii* in microalgal farming seems unsuitable, essentially due to the extremely slow growth rate. By contrast, fast-growing microalgae species belonging to the class of Chlorophyceae appear the most likely to be successful improving the oil yielding of algae mass-culture systems. Compared to *B. braunii*, fast-growing algae offer more promising prospects, even with a lower lipid content. Based on our results, even species with a medium lipid content of 20% of the biomass can be effective, provided a growth rate as high as 2 doubling/day.

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P 64

Biological control activity and molecular characterisation of the Ectomycorrhizal fungus Pisolithus spp. in Morocco

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One collection of fifteen isolates of Pisolithus spp. associated with the Eucalyptus spp. And Quercus suber were sampled on the Maamora forest in Morocco. Their antagonism activity was tested towards five phytopathogenic fungi (Fusarium oxysporum, Helmintosporium maydis, Botrytis cinerea, Armellerea mellia and Curvularia lunata) to select the more preferment isolates. The isolates of Pisolithus spp showed variable behaviour with regard to the pathogen. Direct confrontation between Pisolithus spp and pathogens confirmed the antagonism either in the presence of the ectomycorrizal fungus or in cell free medium. This was attributed to eventual biopesticides substances secreted in the medium. However more investigations are needed to identify the chemical substances and select the most efficient species. Comparison between isolates on the basis of molecular analysis (ITS-RFLP).revealed differences in their patterns. The observed variety could be exploited in integrated programme of controlled mycorrhization in Morocco. On another hand, filtrate issued from ectomycorrhizal fungi culture could be used in posts harved treatments.

P 65

Natural oils in high temperature stable microemulsions containing room- temperature ionic liquids as polar pseudo-phase

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In the current contribution we present a new concept to extend the conventional stability range of microemulsions and to replace conventional oils by biodiesel, a fuel of biological origin.
Ionic liquids are often considered as green solvents, because they are non-volatile and non-flammable. Furthermore, they exhibit high solvent abilities and high thermal stability. We are interested in environmentally friendly microemulsions that exhibit a thermal stability beyond the conventional stability range of microemulsions. We formulated non- aqueous microemulsions with biodiesel as continuous phase, long-chain ionic liquids as surfactants and room-temperature ionic liquids as polar microenvironment. All ingredients show excellent thermal stability. We will demonstrate the effect of the replacement of conventional oils by biodiesel on the phase diagrams. Furthermore, the characterization of these new and environmentally friendly systems will be discussed.
The huge thermal stability range of the microemulsion opens a wide field of potential applications.

P 66

A novel sorbent material for oil spill cleanup

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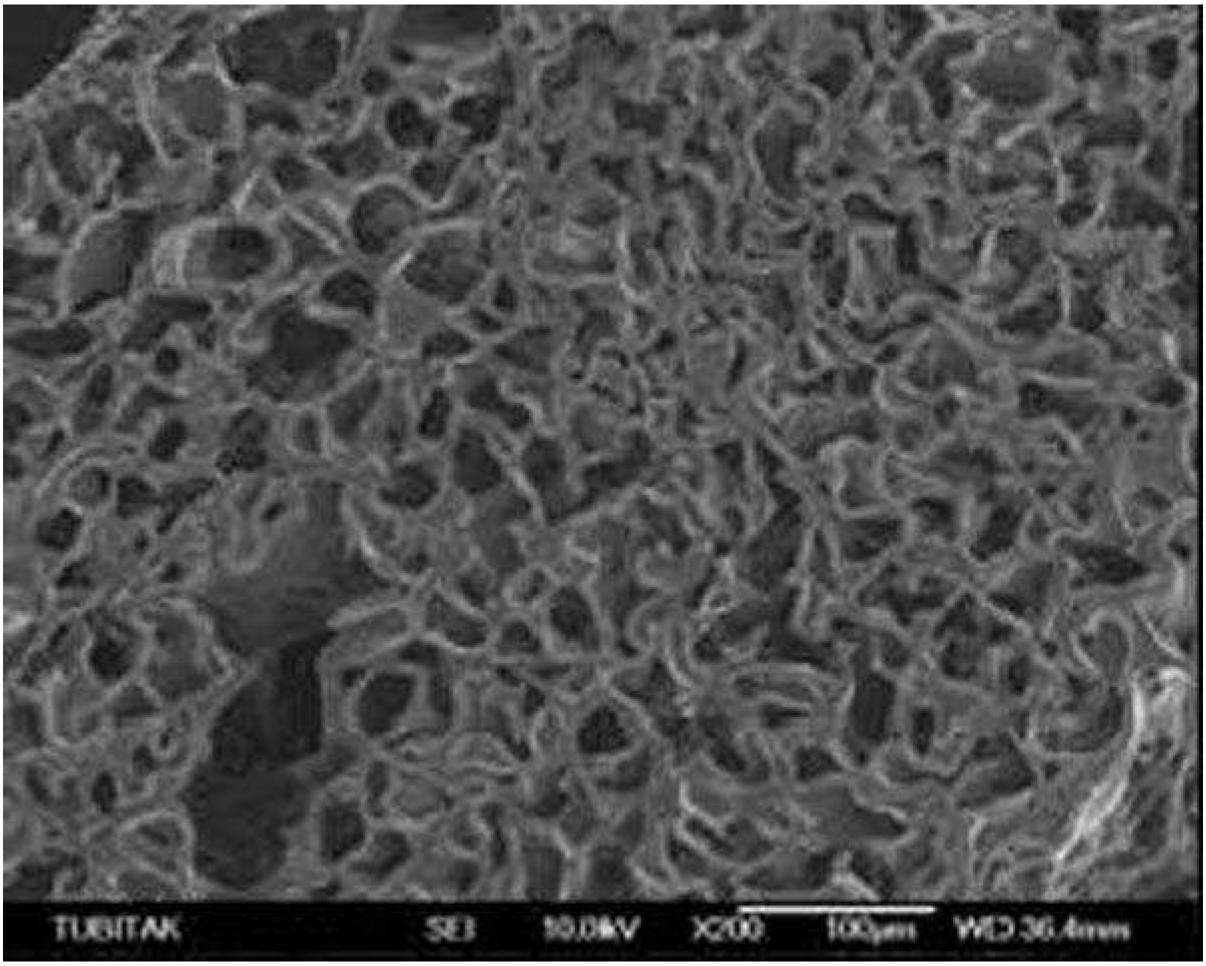
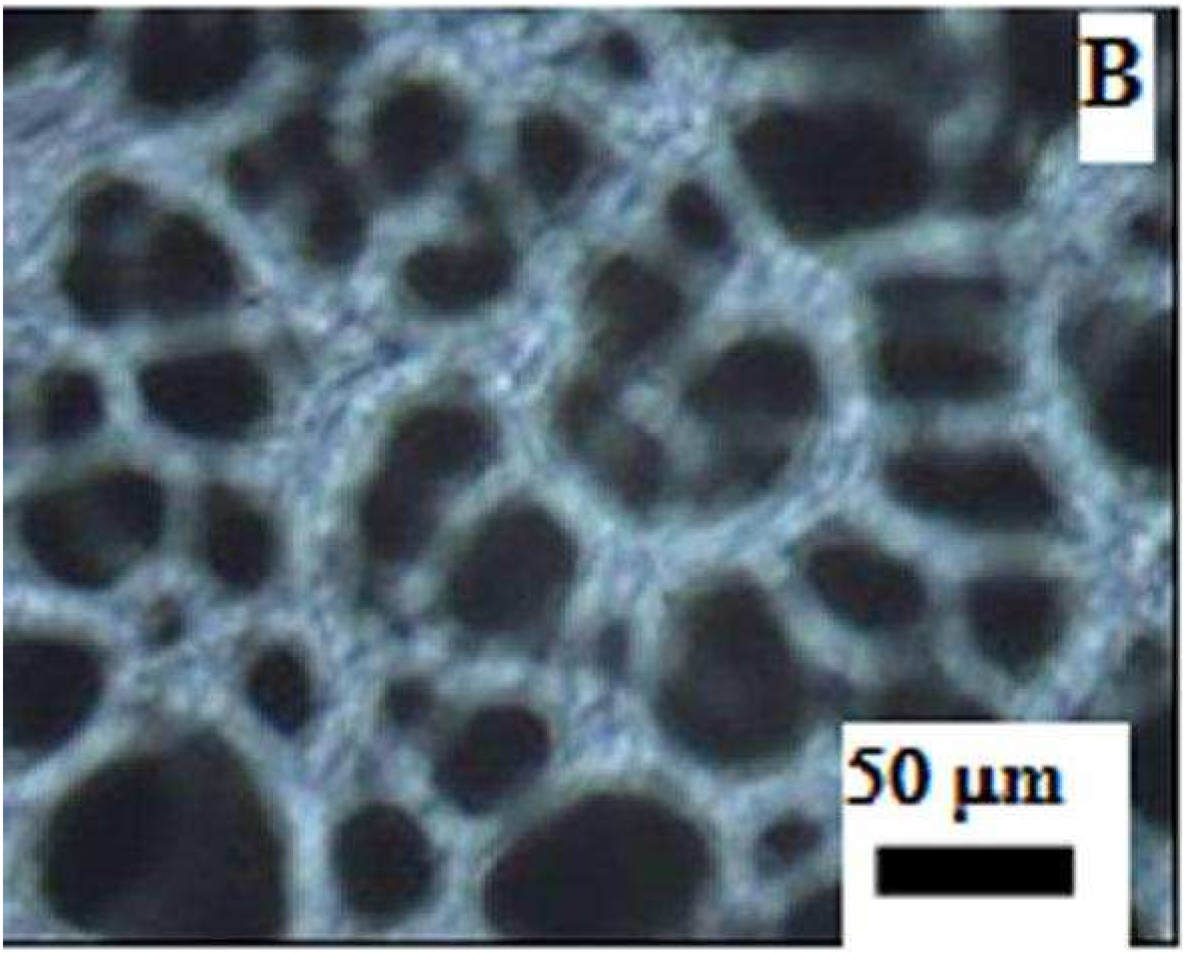
Contamination of seawater by organic pollutants such as crude oil, refinery discharges, industrial and municipial wastewater discharges have been an important environmental issue. For the removal of oil spills from waters, several techniques were proposed. Among the main existing techniques, the use of oil sorbents appears to be the most effective one because of the possibility of collection and complete removal of the oil from the oil spill site.
In this study, a novel sorbent material based on butyl rubber was used for the removal of oil and oil derivatives from waters. The results were compared with those obtained using commercial oil sorbents based on polypropylene. The sorbent materials were prepared by solution crosslinking of butyl rubber using sulfur monochloride as a crosslinker at subzero temperatures, as described previously [1,2]. The oil sorbents thus obtained have a porous structure with irregular large pores of 10 - 100 micrometer in diameter, separated by pore walls of about 10 μm in width with a high polymer concentration, which provide structural support to the material (Figure).



[A]



[B]

[C]

Scanning electron microscopy (SEM, A) and optical microscopy (B) images of the sorbent material. The scaling bar and magnification of SEM are 100 μm and x200, respectively. (C): Images of sorbent materials of various shapes.

The rubber solvent is capable of sorbing selectively crude oil and oil derivatives (20 - 30 g/g) from water surface as well as polycyclic aromatic hydrocarbons (PAH's) from water due to hydrophobicity. More importantly, the rubber sorbents are very tough; they can be compressed up to about 100 % strain without any crack development, during which the total organic pollutant inside the sorbent can be recovered. The high sorption capacity as well as the reusability of the sorbent material after simple squeezing suggests that it can be widely used in oil spill cleanup operations.

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P 67

Zinc complexes with guanidine-pyridine hybridligands: New catalysts for the ring-opening polymerisation of D,L-lactide

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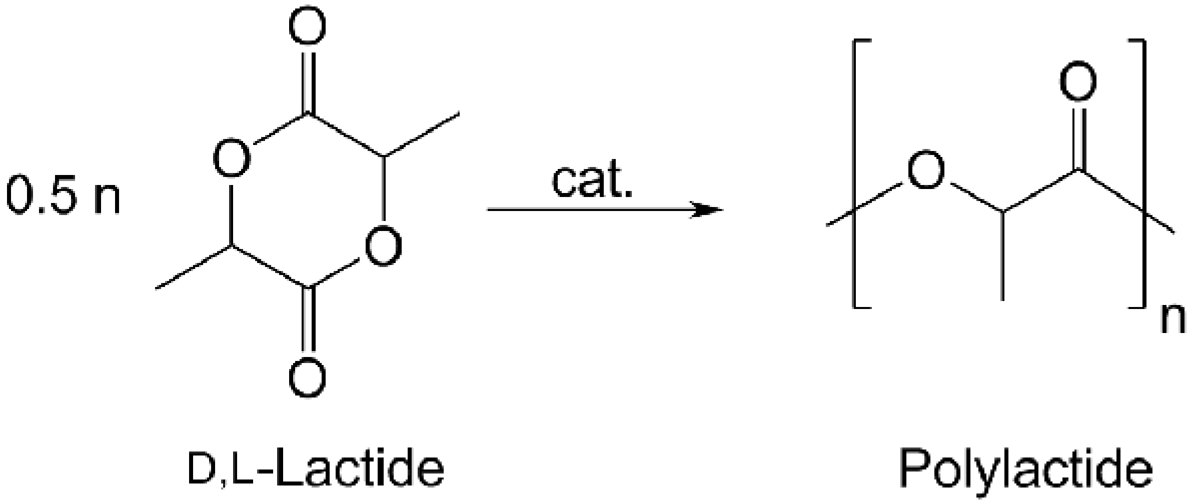
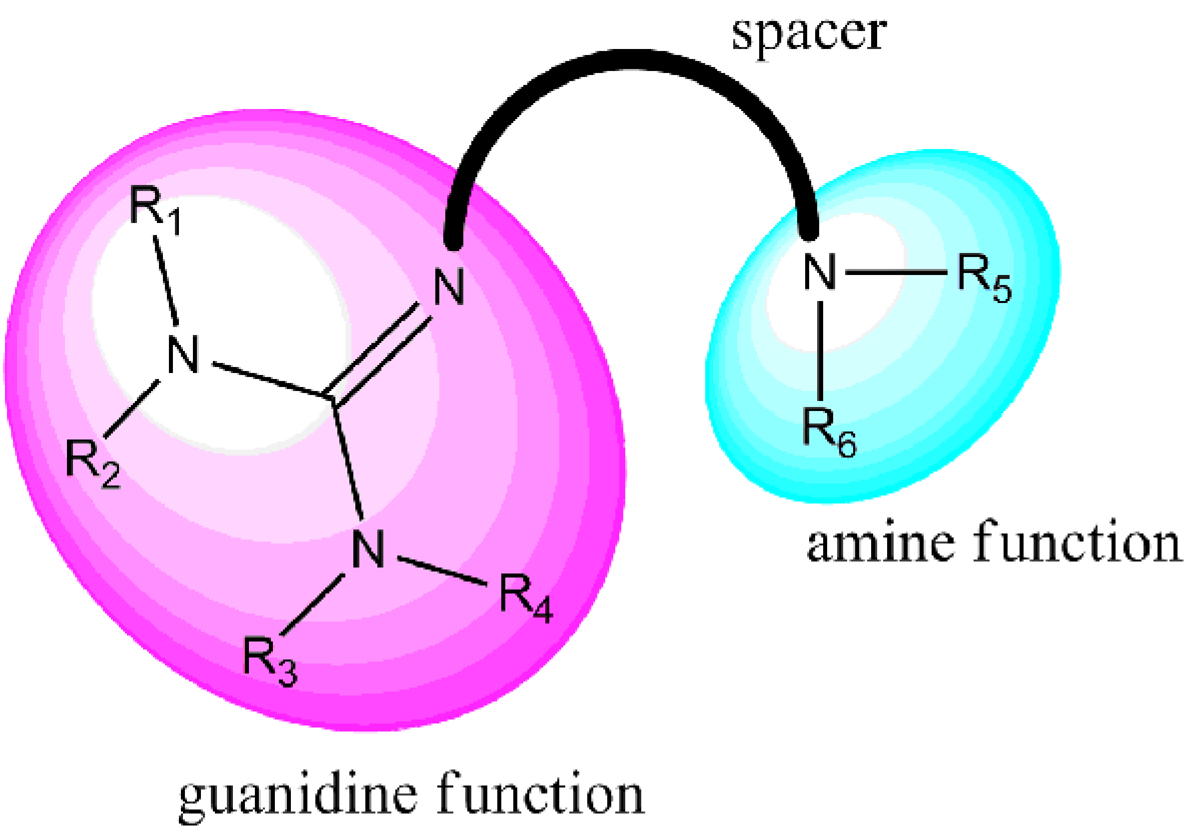
As we reported recently[1] , zinc guanidine complexes show a high potential as active catalysts in the ring-opening polymerisation (ROP) of D,L-lactide. Polylactides (PLAs) have proven to be the most attractive and useful class of biodegradable polyesters among the numerous polyesters studied so far. Due to their favourable material properties as well as their biodegradability and the fact that they can be produced from inexpensive renewable raw materials like corn, sugar beets or even agricultural waste, PLAs are qualified to be a viable alternative to petrochemical-based plastics. They can be either recycled or composted after use and therefore they are CO_2-emission-neutral. Due to the combination of unique physical properties combined with biocompatibility and the ability to degrade both in vivo and in vitro, PLAs possess a great range of applications ranging from widespread use, including coatings, fibres, films and packages, to use in specialised medical and pharmaceutical fields. To achieve high molecular weight, PLA is produced by metal catalysed ROP of lactide, the cyclic diester of lactic acid (Fig. 1).[2]



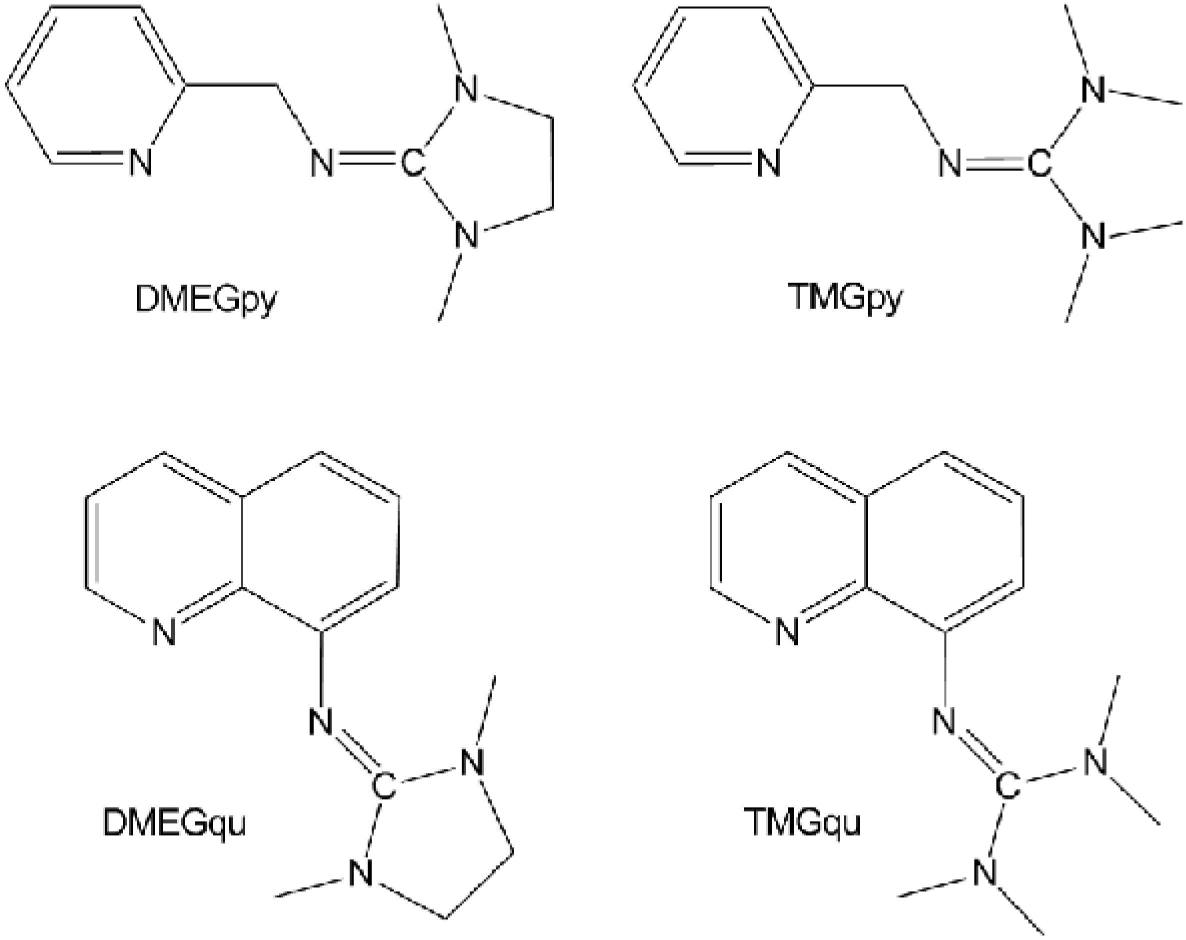
[1]
Figure 1. ROP of D,L-lactide

In order to enhance the polymerisation process to change the polymer from a speciality material to a large-volume commodity plastic, and to replace common Sn-based catalysts, zinc complexes with N-donor-functionalised ligands are used because they are mostly colourless, inexpensive and non-toxic.[2a,b,d] Examples of guanidine zinc complexes have already been reported.[3] It could be shown that some of these possess catalytic activity,[4] which makes guanidines a very promising ligand class leading to catalytically active zinc complexes. Bisguanidines offer excellent donor properties that allow the coordination of metal ions and enable the access of substrate molecules to the metal centre at the same time. A great advantage of bisguanidines is their modular synthesis protocol, which combines different spacer and guanidine groups and thus admits a flexible ligand design.[5] The variation of the ligands allows an optimal adaptation to actual demands. Moreover, the guanidine ligands are strongly related to the essential and ubiquitous amino acid arginine, which is nontoxic. We could demonstrate that zinc bisguanidine complexes show a high potential as active catalysts in the ROP of D,L-lactide and that they possess an acceptable stability.[1]

In order to improve the activity of guanidine based zinc complexes we modified the ligand system by substituting one guanidine function by an amine group. This substitution changes the electronic environment by replacing one “hard” guanidine function by a “soft” pyridine donor and simultaneously the accessibility to the zinc centre by substituting one bulky guanidine by a non-bulky pyridine unit. This guanidine-amine hybridligands combine the excellent donor properties of guanidines with additional coordination space for the pre-coordination of substrates and they also exhibit a modular synthesis protocol and thus admit a flexible ligand design (Fig. 2a).[5b]



[2a]
Figure 2. a) Modular components of hybridguanidines



[2b]

Figure 2. b) Guanidine-pyridine hybridligands[6]

In a first approach, several guanidine-pyridine ligands were synthesised[6] (Fig. 2b) and utilised for the stabilisation of zinc complexes (see Fig.3).[7] These complexes possess a high stability towards moisture and air and show a high catalytic activity in the ROP of D,L-lactide with only few exceptions. PLAs with molecular weights (M_w) up to 176000 g/mol could be obtained.[7] These results represent a significant improvement towards bisguanidine zinc complexes[1] and corroborate our strategy of building up N donor ligands with different donor strengths and substituents with varied steric demands. Within the group of complexes with the same ligand, the activity of the initiator depends on the kind of anionic component of the zinc salt. We found that zinc triflate complexes are excellent catalysts for application in the ROP of lactide. Compared to other systems the guanidine-pyridine hybridligand zinc complexes possess an advantageous combination of properties: they are non-toxic, can be stored on air without loss of activity and give PLAs with good M_w values at industrial conditions.[7] These properties make the guanidine zinc systems an excellent class of catalysts for the ring-opening polymerisation of D,L-lactide. Further investigations will focus on the kinetics of the polymerisation process and a mechanism of the D,L-lactide activation and polymerisation.

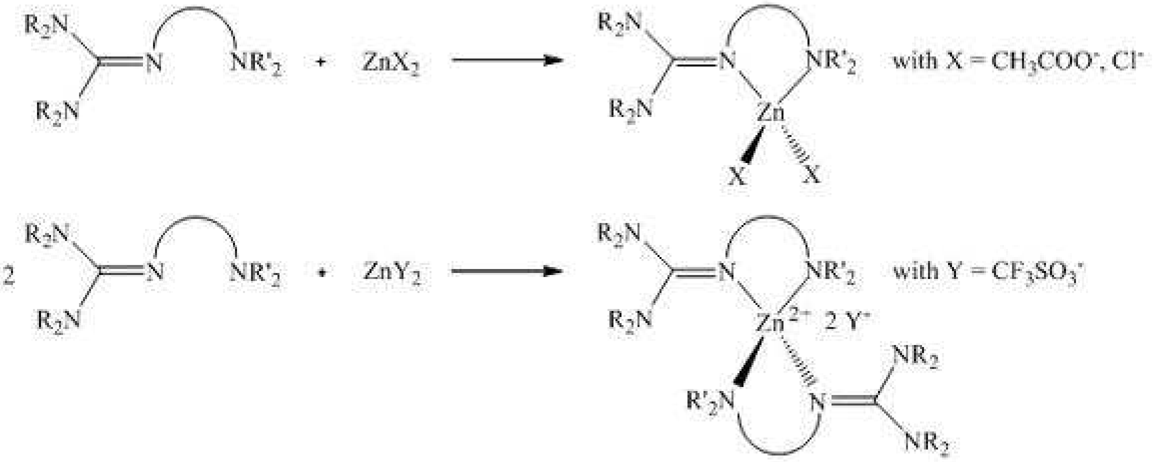


Figure 3. General complex synthesis

[3]

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P 68

One pot iron-catalyzed cross-coupling reactions

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Metal catalyzed-cross-coupling reactions have become an indispensable tool for the formation of C-C Bonds on industry and lab scale. Common cross-coupling-reactions use expensive palladium or toxic nickel catalysts. That remains as a drawback for this kind of reactions. Therefore iron-catalyzed-cross-coupling reactions seem a sustainable alternative for the common cross-coupling reactions. The effectiveness of such coupling reactions in which organic halides are coupled with an nucleophilic organomagnesium species has already been well-tried in the synthesis of different important building blocks and natural products.
In this report we show an operationally simple protocol for the efficient iron-catalyzed cross-coupling reaction of arylhalides with alkylhalides to give substituted arenes in a one-pot procedure under mild conditions. The reaction involves the iron-catalyzed formation of small amounts of the prerequisite organomagnesiumspecies and its fast consumption in an iron-catalyzed cross-coupling reaction with high selectivity. The overall process utilizes a single cheap pre-catalyst (FeCl3) and obviates the need for the handling of large quantities of the hazardous organomagnesium halide.
In order to study the reaction, the influence of amine additives, solvent and reaction conditions onto the selectivityand substrate scope were investigated.

P 69

Environmental assessment in early stages of process development - microreaction technology under rough conditions

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Continuously running microreaction processes offer novel ways of process intensification, in addition to other reactor and process engineering advantages such as process safety or modularity. Due to the characteristics of microstructured devices, esp. due to the highly efficient heat and mass transfer, a considerable reduction of the environmental impact is commonly expected from the implementation of microreaction technology into industrial processes.[1] A descriptive example for this is the environmental benefit of microreaction technology compared to conventional batch technology in the case of highly exothermic reactions like lithium-organic syntheses.[2]
To exploit the inherent benefits of microreaction technology, a new research cluster called Novel Process Windows has been founded by the German Federal Environmental Foundation (DBU). “Novel process windows” mean reactions under rough conditions (high p, T or c) which should be realisable safely in microreactors with improved yield and/ or selectivity. For this purpose, different techniques of providing energy like microwave or ultrasound irradiation or the application of novel solvents (e.g. ionic liquids) are under investigation. But, these improvements resulting from rough process conditions may demand additional energy input as well as intensive process control because of higher safety requirements.
Our aim is to evaluate and compare these new process strategies with conventional methods via an environmental assessment already at R&D stage, in order to guide the decision making process and to show the key issues of ecological improvements. First results have shown that microwave irradiation should only be applied if higher yields are obtainable. Furthermore, the employment of ionic

liquids may be advisable only in case they can be reused.

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P 70

Removal of uranium ions from aqueous solutions by calcium alginate beads

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Uranium is one of the most serious contamination concern because of its radioactivity and heavy metal toxicity arising from the nuclear industry as well as from anthropogenic activities. Excessive amounts of uranium have found their ways into the environment through activities associated with the nuclear industry. Uranium may exist in freshwater environments in a variety of soluble forms, including the dissolved uranyl ion (UO22+) and uranyl complexes with inorganic (e.g. sulphate, carbonate) and organic (e.g. humic and fulvic acids) ligands. There is a need for controlling the heavy metals and radioactive elements emissions into the environment. Biosorption, which uses the ability of biological materials, is a relatively new technology to remove metals from wastewaters.

Alginates are used in the food, textile, cosmetic, paper, and pharmaceutical industries. Also, many recent studies have showed that alginate is an efficient biosorbent of heavy metals and radioactive elements such as Cr, Cu, Zn, Ni, Co, Ag, Au, Am, and Ru as well as non toxic to humans, biodegradable and cost effective. Alginate is the common name given to a family of linear polysaccharides containing 1,4-linked β-D-mannuronic (M) and α-L-guluronic (G) acid residues arranged randomly along the chain. It is one of the main components in brown algae, from which it is extracted. The carboxylic groups are generally the most abundant acidic functional groups in the alginate polymer, and the sorption capacity of algae is directly related to the presence of these metal-binding sites.

In this study, sorption potentiality of uranium ions is investigated using alginate biopolymer beads in diluted aqueous solutions. The ability of prepared alginate beads to adsorb uranium(VI) from aqueous solution has been studied at different experimental conditions of pH, concentration of U(VI), contact time, biomass dosage and temperature. This study also presents and discusses morphological characteristics and analyzes of alginate beads before and after sorption. The percent adsorption (%) and distribution constant (Kd) for uranium ions were 91±1 and 10043±834 mL/g respectively under the optimized experimental conditions.

The sorption results have been subjected to different sorption isotherms, namely Freundlich, Langmuir and Dubinin-Radushkevich. Biosorption equilibrium better fit Langmuir equation than Freundlich equation, besides don’t fit Dubinin-Radushkevich model. The thermodynamic parameters such as variation of enthalpy ΔH, variation of entropy ΔS and variation of Gibbs free energy ΔG were calculated from the slope and intercept of lnKd vs. 1/T plots. The results of this study showed that natural and cheap biopolymer Ca-alginate beads can be successfully used for uranium removal from diluted aqueous solutions.

P 72

Conversion of carbohydrates into 5-hydroxymethylfurfural in carbohydrate melts

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Furan derivatives, such as 5-hydroxylmethylfurfural (HMF) obtained from renewable biomass-derived carbohydrates have the potential to be sustainable substitutes for petroleum-based building blocks used in production of fine chemicals and plastics. Solvents conventionally used for the dehydration of hexoses to form HMF are water, aprotic organic solvents (DMSO), biphasic systems and ionic liquids (ILs). Depending on the solvent system, different undesired side products may be formed (low selectivity) or the whole system shows a rather moderate conversion. Amongst others, the initial carbohydrate concentration is a limiting factor for the efficient preparation of HMF, being relatively low in most systems.

To overcome this limiting factor, we studied the applicability of carbohydrate/urea melts with very high initial carbohydrate concentrations for the selective formation of HMF. Diverse carbohydrate melts were established in our group for the use as solvents in organic transformations, as well as investigated for their physical and chemical properties.

Initial studies screening different melt components with D-fructose revealed that a D fructose:N,N´-dimethyl urea melt (60/40 w/w) gave the best HMF yields with different catalysts tested. Besides the tested Lewis acids ($FeCl_3$, $AlCl_3$, $ZnCl_2$, $CrCl_2$, $CrCl_3$), the acidic ion exchange resin Amberlyst gave significantly better yields of HMF without the detection of the rehydration products levulinic and formic acid. Acceptable yields for the DMU/carbohydrate melt were obtained after 2 h at 110 ℃ in 30-40 %. Further, the use of organic acids like malonic and maleic acid as melt components instead of DMU was investigated in order to reduce the pH value, due to the accelerated dehydration of fructose, which is reported to take place under very acidic conditions (The fructose/DMU melt has a pH value of 6-7).

The selective route via a DMU/carbohydrate melt is currently addressed, with the purpose to increase the still low conversion of D-fructose to HMF.

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P 73

Catalytic reductions of dimedone

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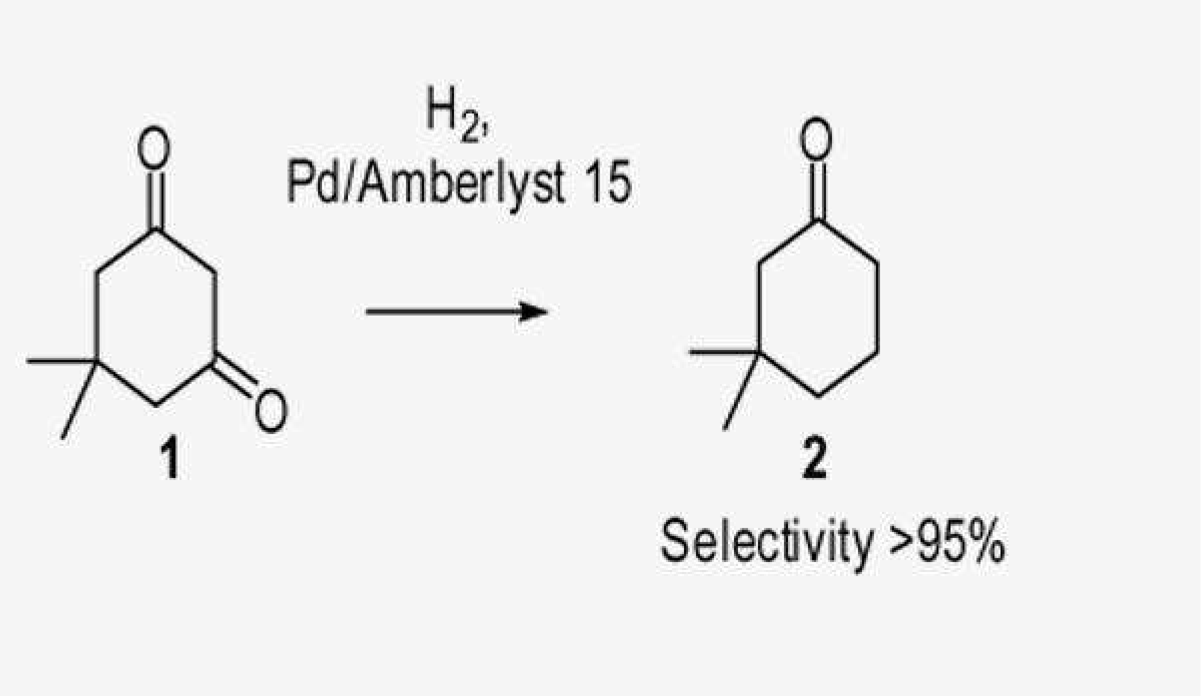
In the area of fine chemicals synthesis, research and development aiming at efficient catalytic procedures is one of the main topics in academia and industry [1]. Heterogeneous catalyzed hydrogenation reactions fulfil the criteria of *Green Chemistry* in terms of atom economy, waste formation, and energy consumption [2].

The selective hydrogenation of dimedone **1** to the corresponding monoketone **2** provides an interesting access to synthetic building blocks for fragrance and pharmaceutical industry.



[graph1]

Dimedone **1** can be reduced in the presence of H2 and a catalyst (Pd/C or Pt/C) towards the monoketone **2**. Described procedures for the reduction are carried out in a carboxylic acid as solvent with addition of catalytic amounts of strong acids like sulfuric acid [3]. For industrial purpose, such conditions rule out their use in standard stainless steel reactors and require acid resistant equipment. Frequently encountered by-products in the hydrogenation are alcohols and various products of aldol condensation.

In this communication we present our results obtained in the reduction of **1** to **2** over palladium and Amberlyst 15. Advantages of the new catalytic procedure are the high catalyst activity, selectivity, less by-product formation, by-passing the presence of acid, and less salt formation due to omitting neutralization. Using a substrate/catalyst ratio of >100 compound **2** can be synthesized in 97% yield at full conversion. Recycling of the catalyst has been shown to be feasible for several times without decrease in activity and selectivity.

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P 74

Methanetrissulfonic acid: A highly efficient strongly acidic catalysts for vitamin E synthesis

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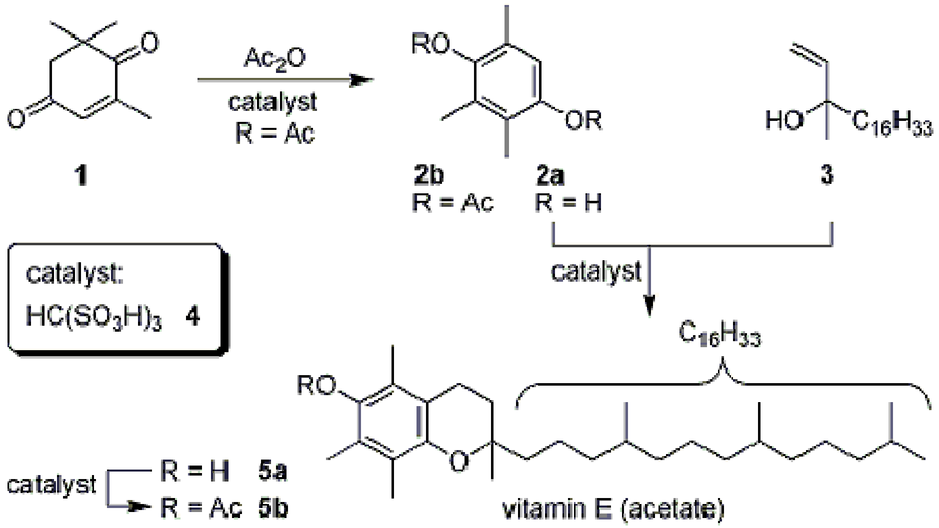
The development of catalytic processes is a fundamental issue in the field of vitamins and fine chemicals industry [1]. In the course of our activities dealing with key-steps of industrial syntheses of vitamins, e.g. economically important vitamin E (acetate) **5a**,**b** [2], we were interested in developing environmentally benign alternative procedures for production. One particular aspect was the search for efficient catalysts acting in a variety of reactions, in order to replace conventional Lewis or Brønsted acids like boron trifluoride, aluminum chloride, zinc chloride, hydrochloric acid, or sulfuric acid.



[Scheme]

The compound methanetrissulfonic acid (**4**) has been prepared for the first time 140 years ago [3]. Although this acid is readily available, there are only few literature reports on its use in chemical transformations.

Examples of its application are Wagner-Meerwein rearrangement (**1**→**2b** [4]), Friedel-Crafts alkylation and ring closure (**2a**+**3**→**5a** [5]), as well as acylation reactions (**5a**→**5b** [6]). Application of catalyst **4** in truly catalytic amounts (0.04-1.0 mol%) resulted in highly selective transformations and yields over 95%.

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P 75

Magnetic TEMPO

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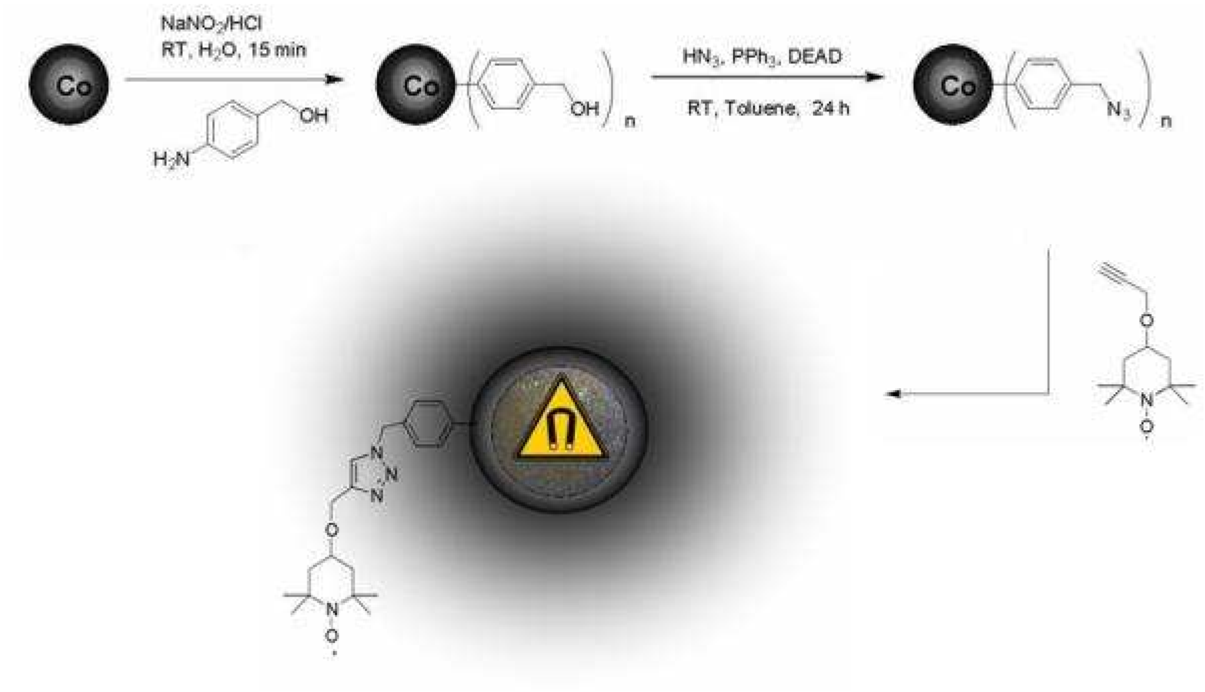
The selective oxidation of primary and secondary alcohols into the corresponding carbonyl compounds is one of the most important transformations in organic chemistry. Common reagents for these oxidations are usually toxic chromium(VI) salts in stoichiometric amounts causing a severe environmental problem. Therefore, the development of systems displaying high atom efficiency using comparatively harmless oxidants such as oxygen, peroxide or hypochlorite is desired. The stoichiometric oxidation of primary alcohols to the corresponding aldehydes by the oxoammonium cation was first reported by Golubev and coworkers in 1965. The oxoammonium cation could also be generated from TEMPO in situ using single oxygen donors such as sodium bromite, persulfate, or sodium hypochlorite.

The stable nitroxyl radical 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) exhibits benign properties such as low toxicity and a reversible redox behavior which motivated its application in combination with these diverse primary oxidants. Especially the protocol developed by Anelli et. al. in 1987, using household bleach buffered at pH 9 at 0℃ in combination with 10 mol% sodium bromide and 1 mol% 4-methoxy-TEMPO in dichloromethane/water is widely applied in organic synthesis. Both primary and secondary alcohols are converted to carbonyl compounds in high yields, even in large-scale operations. Whichever oxidant is used, product isolation and catalyst recovery remain key issues. Moreover, although low catalyst concentrations are required (typically 1-2.5 mol%), TEMPO is quite expensive. Consequently, it is highly desirable to separate and reuse the catalyst after the oxidation reaction. Several groups have addressed this problem by anchoring TEMPO to solid supports such as polymers, silica or by entrapping TEMPO in a silica sol-gel whereas nanostructured materials are rarely seen.

We expected carbon coated cobalt nanoparticles to be a highly stable support which in addition shows excellent ferromagnetic behaviour, allowing quick and easy separation of the catalyst from the reaction mixture. The regular shape and high surface area are tantalising properties of nanoparticles but sight may not be lost of long-term stability and economic sense in synthesis. The copper(I)-catalyzed alkyne/azide "click" reaction is an excellent strategy for the surface modification combining high efficiency and wide tolerance of functional groups under mild reaction conditions. Therefore, azide-functionalized C/Co-nanopowder proved to be an excellent support for TEMPO modified with a terminal alkyne moiety. The catalytic activity of the resulting heterogeneous catalyst was shown to be equal to TEMPO on conventional supports.



[TEMPO immobilised on magnetic C/Co-Nanoparticles]

P 76

Production of high value fragrant wood and olfactory active compounds from tropical thymelaceen

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Eaglewood (idn. gaharu) is the most highly valuable resinous wood used for incense, perfume and traditional East-Asian medicines and is derived from tropical trees of the family of Thymelaeaceae. High consumer demand, particularly from Middle Eastern and Asian markets, combined with a significant decreased supply as a consequence of non sustainable harvest has raised up prices for top grade agarwood to over 10.000US$/kg. Agarwood can not generate in the healthy wood tissue as more that it is an induced resinous product of wounding due to herbivory, wind chill or fire damage and subsequent opportunistic infection by facultative fungal pathogens. However, the underlying physiological and particularly the molecular mechanisms of the stress induced agarwood formation in the wood of the Thymelaeaceae trees are still unknown. The main sources of the traded Indonesian Gaharu consist of the aromatic resinous wood from natural populations of the genus Aquilaria and Gyrinops. The random process of agarwood formation by wounding and opportunistic infection implies that only a small proportion of the wild Aquilaria and Gyrinops trees harbors the desired fragrance wood. Since Indonesian agarwood collectors tend to harvest the majority of trees in natural ecosystems, regardless if they contain the aromatic resin, the average population size of both genus decreased below 0.6 trees/ha and hence are hence are listed on the IUCN (International Union for Conservation of Nature and Natural Resources) red list of threatened species (IUCN 2006).
Phytochemical investigation on constituents and the quality of Indonesian agarwood derived from A. microcarpa and G. versteegii with respect of distinct metabolic profiles or single high value compounds has never been done. The recent discovery of discrete odor types suggests, that diverse metabolic pathways and metabolic profiles are elicited in Aquilaria and Gyrinops due to artificial inoculation with different opportunistic Fusarium species including Fusarium oxysporum and Fusarium lateritium. Furthermore, the metabolic contribution of the involved distinct Fusarium species on terpenoid pattern and quality of Agarwood has never been elucidated. Systematic phytochemical analysis offers therefore the possibility to detect unknown patterns of fragrance compounds or new single high value compounds with superior structural and olfactory properties.
Supported by the Government of Indonesia & Germany a bilateral F&E research network on Bioprospecting of Indonesian Agarwood has been established in order to

a) assess the terpenoid quality of actual traded Indonesian agarwood derived from A. microcarpa and G. versteegii,
b) establish a suitable host pathogen combination to produce high quality agarwood with special respect to olfactoric superior constituents,
c) develop a early screening technique leading to a marker assisted selection of elite mother plants and
d) develop biotrechnological strategies via plant cell and tissue engineering techniques to produce single, high quality secondary metabolites from Gyrinops and Aquilaria ssp.

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Water as catalyst for microwave-assisted isomerizations of pinenes?

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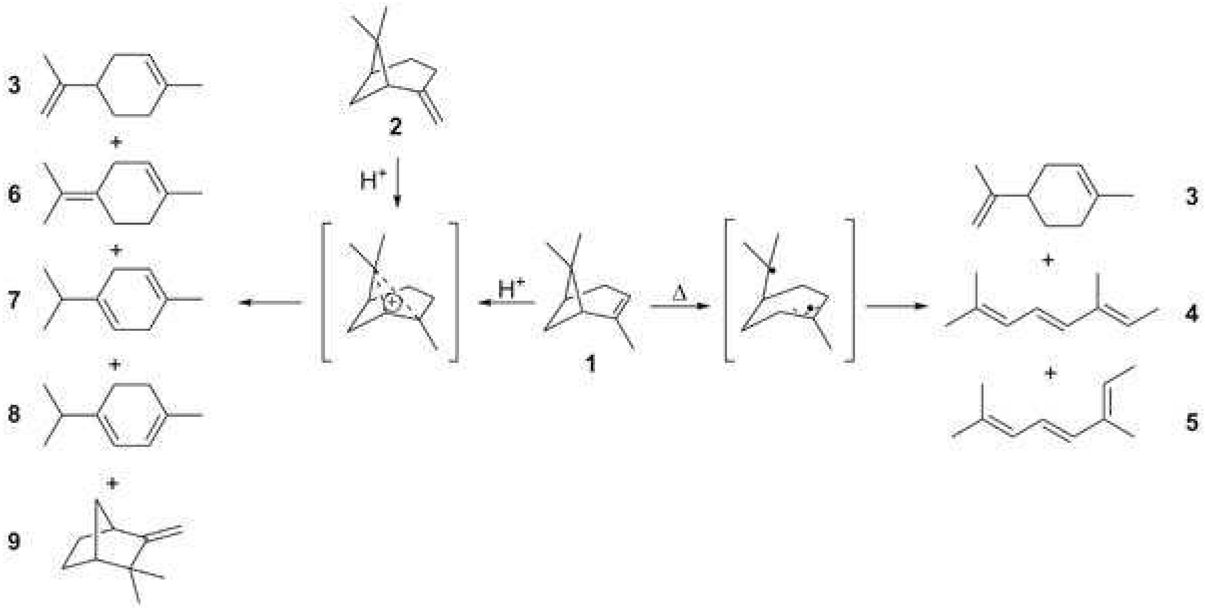
The isomerization of monoterpenes and monoterpenoids (α-pinene, β-pinene, camphene) is an important reaction for the production of various fine and specialty chemicals for instance: flavors, fragrances or pharmaceuticals. Depending on the mode of activation (thermolysis, photolysis or acidolysis) those rearrangements lead to different products. Whereas thermal or photochemical activation yielded worthy products, whose formation can be explained by application of the Woodward-Hoffmann-Rules of orbital conservation, reactions in presence of acidic substances (mineral acids, organic acids, Lewis acids, acidic sites on heterogeneous surfaces) result in the formation of strongly rearranged products. For example the acid-catalyzed ring-opening of α-pinene with mineralic acids yields *p*-menthadiene-type products (limonene, ….) and performing of the reaction in presence of zeolithes leads to the formation of camphenes.
Normally, those monoterpenes do not undergo isomerization if the reaction is performed in pure water without the presence of any other catalyst. Recently it was reported that water on heating to subcritical conditions changes its pK_S-value allowing for catalyzing reactions normally needing an acidic catalyst

or additive to precede with acceptable conversions and selectivities (e.g. hydration, ester hydrolysis, pinacol rearrangement).[1] Based on these findings water was used in the present study as solvent for isomerization reactions of pinenes (α-pinene, β-pinene) under subcritical conditions yielding *p*-menthadiene derivatives (terpinene, limonene). Reactions were carried out in high-pressure vessels mounted in the microwave system *Synthos 3000* from Anton Paar within a temperature range of 200-300 ℃ and pressures up to 80 bar. Results will be presented studying the influence of substrate-to-water ratio, reaction time, type of substrate, reaction temperature and non-acidic additives added to the solvent (NaCl). The main isomerization products yielding from treatment of α-pinene (**1**) and β-pinene (**2**) under microwave irradiation in the presence of subcritical water are shown in Scheme 1.



[1]

SCHEME 1: Rearrangement products from isomerization of α-pinene (**1**) and β-pinene (**2**) in presence of subcritical water.

Under the reaction conditions applied, **2** furnished mainly *p*-menthadiene-type rearrangement products: limonene (**3**), terpinolene (**6**), γ-terpinene (**7**), and α-terpinene (**8**). Also small amounts of camphene (**9**) and alcohols resulting from addition of water (fenchol, terpineol) have been identified in minor amounts. The formation of these products is in accordance with other studies using either mineralic acids or proton-enriched heterogeneous catalysts (zeolithes).[2] Results can be rationalized assuming that the reaction passes through non-classical carbocations.

Contrarily to **2** the isomerization of **1** afforded beside rearrangement products resulting from acid-catalyzed reaction (**3,6-9**) also $C_{10}H_{16}$-isomers apparently yielding from thermal isomerization: **3**, 4Z,6*E*-alloocimene (**4**), and 4Z,6Z-alloocimene (**5**). The formation of theses products is due to the formation of a biradical intermediate (cf. Scheme 1) which undergoes [1,5]hydrogen shift to form **3** or further ring-opening yielding the acyclic isomers **4** and **5**.[3] Carbocation intermediate is the same as in case of **2** but an isomerization of **2** leading to **1** was not observed.

The different behaviour of the two pinenes whose isomerization behaviour has been investigated is due to their different behaviour in thermal isomerization reactions. The product selectivity of products resulting from acidolysis (without **3**) is the same for **1** and **2**. It is well known that 1 is the more reactive counterpart regarding thermal isomerization reactions. The activation energies for the gas-phase rearrangement are 170 and 186 kJ mol^{-1} for **1** and **2**, respectively.[3b] Therefore treatment of **1** under the reaction conditions applied herein afforded both products from thermal and acid-catalyzed rearrangement.

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P 78

Microwave-assisted hydrogenations in an innovative microwave system

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In the last years the application of catalytic methods has increased in order to reduce production costs, minimize waste and improve selectivities. The liquid-phase hydrogenation of unsaturated compounds is an important step in the preparation of fine chemicals in different areas like pharmacy, human and animal nutrition or the flavor and fragrance industry. Predominantly heterogenous gas-phase hydrogenations or homogenous reactions in liquid-phase carried out in industrial sector. The combination with microwave techniques allows new aspects and applications in chemical industry. The use of microwave heating has attracted remarkable interest in the last 10 years. Advantages of this alternative energy-form are a dramatically cut of reaction times, an increase of product yield and purity. Several reaction types (e.g. C-C coupling reactions, oxidations, hydrogenations) can be carried out in the very different microwave systems.

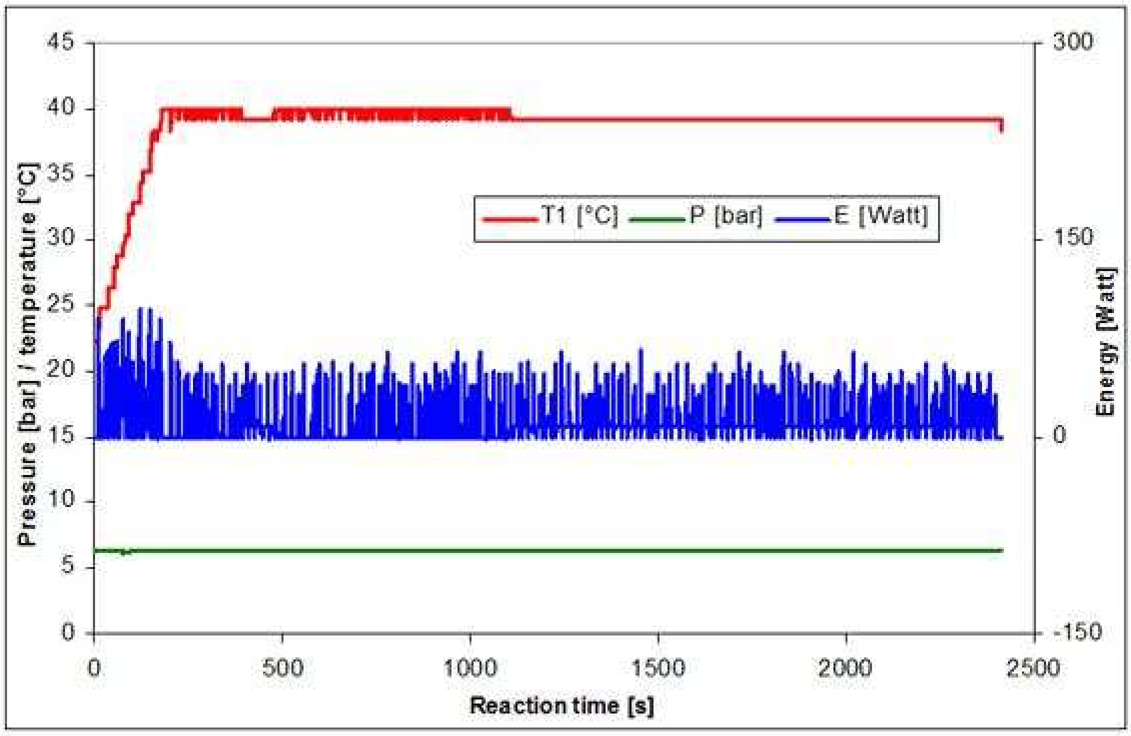

Figure 1: Temperature-pressure-energy diagram of a hydrogenation reaction *[1]*

The tested innovative microwave system was a so called QRS-reactor.[1] This system is applicable for reactions under increased pressure and temperature. The advantages of the reactor system are the easy handling and the possibility of versatile in different areas of chemical synthesis. Further the sampling from the reaction mixture and the direct gas inlet in the reaction mixture are feasible. The combination with a multimode microwave apparatus allows for simple and permanent control of the reaction parameters: pressure, temperature, and energy input. Figure 1 shows a typical temperature-pressure-energy diagram of a complete hydrogenation reaction using dihydrogen as reducing agent. The temperature is measured by an ATC-CE sensor and the pressure by a pressure sensor directly in the reaction vessel. Additionally, the QRS-reactor allows safe and effective application of gaseous hydrogen into the reaction mixture thus preventing mass-transfer limitations. With the described system it is possible to perform chemical reactions under a maximal pressure of 10 bar within temperature ranging from room temperature to 200 ℃ .

The liquid-phase hydrogenation was the chosen as model reaction for the new reactor. Different parameters like reactant, catalyst, temperature, pressure and gas in- and through-put were investigated. Hydrogenation of benzalacetophenone (**1**, Figure 2) in ethyl acetate with dihydrogen as reducing agent was used as sample reaction. **1** is an interesting compound because of its three different types of double bonds: alkenyl, aromatic, and carbonyl. As catalyst Pd impregnated on porous glass (Pd/SiO_2) was used due to its thermal and chemical stability, as well as with respect to its simple preparation in only a few straightforward steps.[2]

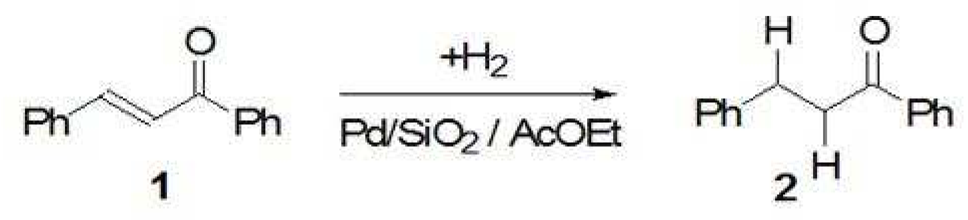

Figure 2: Hydrogenation of benzalacetophenone (**1**)

[2]

The results of hydrogenation experiments with **1** furnished one product only: 1,3-diphenylpropanone (**2**, Figure 2). The alkenyl carbon-carbon double bond was reduced chemoselectively whereas a hydrogenation of the carbonyl group or of the aromatic double bonds was not observed under any reaction conditions tested (max. 8 bar, max. 50 °C) . In summary, the novel microwave reactor is a nice tool for the safe and easy performance of microwave assisted hydrogenation reactions using dihydrogen as reducing agent.

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Green photooxygenations - the synthesis of quinonoid fine chemicals

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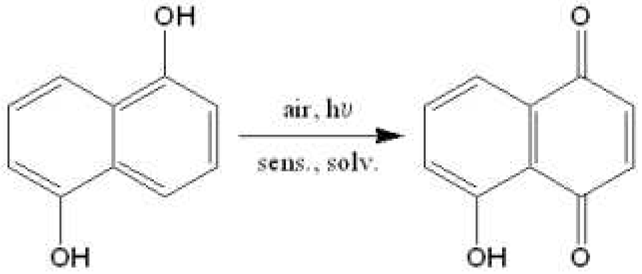
With recent concerns regarding global warming and climate change it is no surprise that the chemical industry has expressed great interest in sustainability. As a result, environmental issues are of much importance, leading to a revolution in the field of green chemistry. The Principles of Green Chemistry [1] give chemists guidelines for sustainable chemistry for the future. With these principles in mind we have looked at the synthesis of quinonoid fine chemicals and introduced green concepts to these syntheses.

Traditionally, 1,4-naphthoquinones are prepared by oxidation with strong oxidizing agents, which tend to be quite hazardous (e.g. chromic acid, thallium trinitrate or peracetic acid). Early photochemical methods reported the synthesis using methanol, acetonitrile or halogenated solvents [2]. However, we have shown that use of longer chain alcohols (*i*-propanol and *t*-amyl alcohol) or microemulsions provide a safe and convenient alternative to these solvents. In addition, we have looked at the use of sunlight as a light source, thus reducing the energy demand of the synthesis.

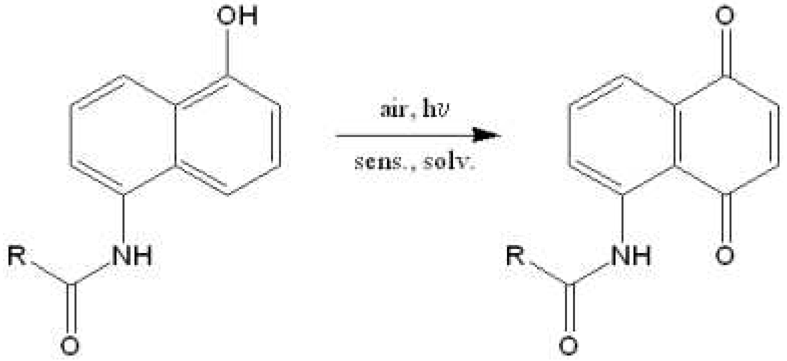
[Figure 1: Set-ups for solar synthesis]

To date we have used the synthesis of 5-hydroxy-1,4-naphthoquinone (Juglone) from 1,5-dihydroxynaphthalene as a model reaction (Scheme 1).



[Scheme 1: Juglone synthesis]

Following the success of Juglone preparation via solar photooxygenation [3], we looked at analogs of this compound, with a particular focus on amide derivatives (Scheme 2).



[Scheme 2: Synthesis of amide deritatives]

Investigations have shown that the synthesis of 5-amido-1,4-naphthoquinones is feasible by solar photooxygenation of the corresponding 5-amido-1-naphthol, both in artificial and solar irradiation conditions. In addition, solar irradiation leads to higher yields in all cases.

To investigate the use of microemulsions in photochemical processes we used the synthesis of Juglone as a model reaction. Microemulsions of water-ethyl acetate (with sodium dodecyl sulfate as surfactant and *n*-butanol or *t*-amyl alcohol as cosurfactant) were optimised. These were shown to possess excellent solubising ability for the substrates and photooxygenation of 1,5-dihydroxynaphthalene was achieved. In addition, various sensitisers were used (Rose Bengal, Methylene Blue and Tetraphenylporphyrin (TPP)), with TPP sensitisation producing the highest yields of Juglone. This demonstrates that microemulsions provide a green alternative to the halogenated solvents commonly required when using TPP.

We would like to acknowledge the Irish Research Council for Science, Engineering and Technology (IRCSET), Science Foundation Ireland (SFI), the Environmental Protection Agency (EPA) and DCU for financial support for this work.

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“Microphotochemistry” - a new, resource-efficient photochemical synthesis tool

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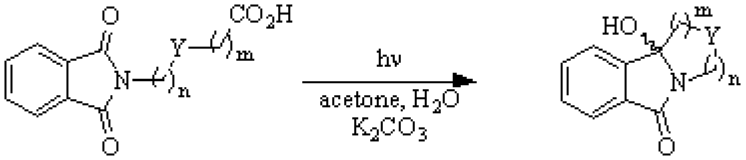
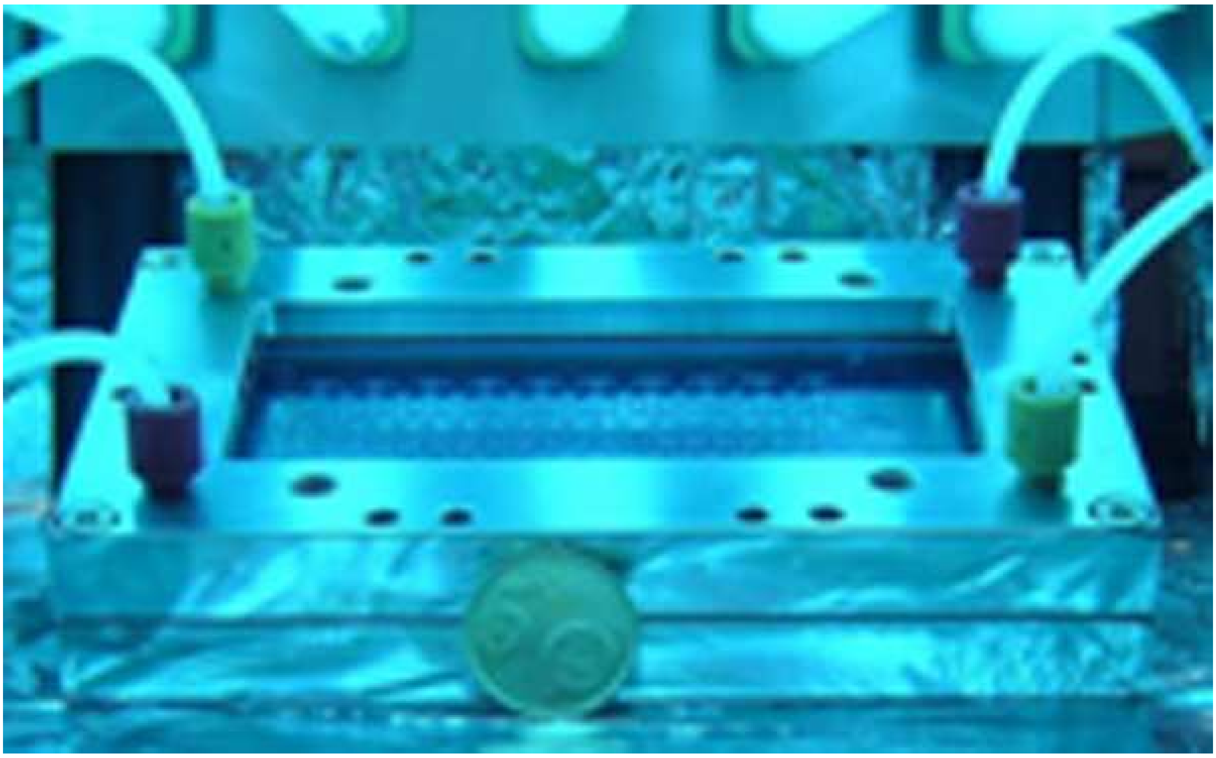
Green chemistry has become increasingly important in recent years just as the need for sustainable processes is becoming more apparent in our everyday lives. There are 12 principles of green chemistry[1] which promote concepts such as the use of safe, environmentally friendly solvents, energy efficient processes and minimising waste produced. Recently, microreactors have emerged as powerful and efficient devices in organic synthesis. Miniaturisation additionally reduces the reaction scale and consequently the consumption of reagents and solvents by several orders of magnitude and makes microchemical devices advantageous for green chemistry.[2] Likewise, photochemistry is regarded as a green technology since it involves photons as 'green reagents'.[3]

As a result, we have recently turned our attention towards micro-photochemistry, which due to its very nature enables us to obey these processes. There has been increased interest shown in this area as it can be utilised for a wide range of photochemical applications.[4],[5]

Over the last decade we have intensively studied photodecarboxylation reactions involving phthalimides.[6] We have thus examined selected decarboxylative addition and cyclisation reactions **[Figure 1]** in a *dwell device* microreactor **[Figure 2]**. Several reactions have been carried out with a view to optimising the reaction conditions and it was found that as we decreased the flow rate and thus residence time, conversion rates increased.



[Figure 1]



[Figure 2]

We have based this particular study on a number of model reactions that have already been carried out on a larger multigram-scale with a novel 308 nm XeCl Excimer lamp.[7] The idea is to perform these reactions on a conventional scale (< 250 mg) in a Rayonet chamber reactor and on a microscale in a dwell device, using lamps with a wavelength centred at 300 nm for both, and ultimately to compare the results with those from the large-scale Excimer runs. The goal is to unambiguously prove that environmentally friendly micro-scale processes are on a par with conventional processes if not better.

Acknowledgements: This research project is supported by Science Foundation Ireland (SFI) and the Environmental Protection Agency (EPA).

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Organic synthesis in hydrothermal salt solutions

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Supercritical water has recently made its way into organic synthesis as environmentally friendly solvent, due to its low dielectric constant acting similar to conventional organic solvents. In addition, the high dissociation constant stands for an increased proton concentration which provides a potential medium for acid-based catalyzed reactions. [1,2,3]
However, supercritical conditions mean working at temperatures above 374℃ and pressures over 218 atm. This work, thus, attempted to lower temperatures into regions of 150-200℃, where the inherent pressure can be handled more easily. In connection with this, we investigate on the addition of inorganic salts as potential catalytic substance to raise the yield. A systematic study of the effect of concentration and the nature of the ions is currently under way.
For a model reaction, alcohols have been elected, in an attempt to shed light into the reaction pathway of hydrothermal carbonization of biomass [4]. In this area, the focus lies in defunctionalization of these complex molecules (mainly the removal of hydroxyl groups), to get to basic carbohydrates needed in industry. Besides the normal dehydration and isomerization, the hydrothermal salt solution was able to promote pinacol rearrangements, aldol condensations, Diels Alder reactions, and to a small amount even dehydration of the alcohol group.

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Green synthesis of p-menthane-3,8-diol from Eucapyptus citriodora: application for repellent product

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A simple and efficient method has been developed for the synthesis of p-menthane-3,8-diol (PMD), a well known repellent active against mosquitoes, from Eucalyptus citriodora essential oil (origin China) by treatment with citric acid or sulfuric acid in a biphasic medium (water/essential oil). The Eucalyptus citriodora oil contains as main components citronellal (74.21%), citronellol (5.95%), isopulegol (4.68%), neoisopulegol (2.25%) and α-pinene (2.10%). As an example, an emulsion containing an aqueous solution of 7% citric acid led at 50℃ for 15 hours conducted to 89% conversion of citronellal to PMD with a selectivity of 83%. A repellent study in a bioassay with Aedes aegypty mosquitoes was performed on the reaction products. A long lasting protection time on human volunteers reached 286 minutes compared to 22 minutes with the pure essential oil.

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Green chemistry versus sustainable development and scientific progress: The example of Cleland's reagent production

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Chemicals are part of modern life and are present in all spheres of human life. They are used as pharmaceuticals, food additives or fertilizers among others and they may contribute to our high life expectancy, economic prosperity and environmental health. Nevertheless, the waste problem in chemicals manufacture is readily apparent. Enormous amount of waste, comprising inorganic salts are formed in most of chemical reactions or in subsequent neutralizations and other work-up steps. Green chemistry should lead to efficient use of raw materials, waste elimination and the avoidance of the use of toxic and/or hazardous reagents and solvent in the manufacture of chemical products.
Besides other compounds, Cleland reagent is a good example of typical chemical production. This reagent also called Erythro isomer of 2,3-dihydroxy-1,4-dithiolbutane (CAS 6892-68-8) or 1,4-Dithioerythritol (DTE) is used intensively in biotechnological industry as antioxydant, denaturating reagent or reducing agent. Its production represents an excellent example of the problems encountered in the chemical industry. It involves multi-step synthesis with three distinct stages. The E factor which varies between 35 to 150 depend on the recycling rate of solvents, quantity of salty water used or acceptable degree of purity among others. The renewable starting material Meso-Erythritol produced naturally from seaweed, mosses and certain grasses is converted to DTE with a yield of 26.5±1.37.
The aim of this study is to rethink Cleland's reagent production using the different concept: green chemistry, sustainable development and scientific progress. From the data collected during 15 years in a small swiss company, which clearly represents a production unit in the chemical industry, the advantages and disadvantages of DTE manufacture are analyzed using these three general concepts. This analysis clearly demonstrates that green chemistry represents the best catalyst to make cleaner (or greener) this production.

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New Co(III) mixed ligand complexes effect on the germination and root length of wheat

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Co(III) mixed ligand complexes derived from catechol(L1) as primary ligand with 2-aminopyridine(L2) and 2-aminobenzo thiazole(L3) as secondary ligands have been prepared and characterized by conventional techniques, including; elemental analysis, electron paramagnetic resonance, infrared electronic spectra, molar conductivity and thermogravimetric analysis. The elemental analysis data display the formation of 1:1:1[M:L1L2] and 2:1:1[M:L1:L3] complexes. The electron paramagnetic resonance spectra of the complexes reveal the oxidation of Co(II) to Co(III) which has a diamagnetic phenomena and support the their geometrical structures. The infrared spectral data show the chelation behavior of the ligands towards metal ion which is through OH, -C=N and -NH2 groups. The electronic spectral results display the $\pi\rightarrow\pi^*$ (phenyl rings), $n\rightarrow\pi^*$ (NH2 and -C=N) and suggest an the presence of an octahedral structure. The molar conductivity reveals the non-electrolytic nature. The thermogravimetric analysis data of the complexes display the existence of hydrated and coordinated water molecules. The effect of CoCl2.6H2O, catechol, 2-aminopyridine, 2-aminobenzathiazole and their complexes on the germination and root length of wheat seeds were evaluated at different concentrations. The prepared complexes were found to exhibit enhanced activity on germination and root length compared to ligands and metal ion.
Key words: catechol, 2-aminopyridine and 2-aminobenzothiazole, mixed ligand complexes, Biological activity.

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Case studies on the development and application of in-Silico techniques for environmental hazard and risk assessment (CADASTER)

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Some 30 000 existing substances are to be assessed through the REACH process within a proposed time-window of eleven years. The REACH proposals advocate the use of non-animal testing methods. However, so far the use of non-testing methods in the European regulatory context is quite limited and fragmented. Reasons include the lack of distinct application criteria and guidance, and the fact that quality and uncertainty of models developed in the QSPR field, including validation and applicability domain of models, frequently is not addressed rigorously and remains a difficult issue.[1-3] The internationally agreed application criteria and guidance, issued by Organisation for Economic Co-operation and Development (OECD)[4] are aimed to improve this situation.

Our FP7 funded project CADASTER (2008-2012) aims at providing the practical guidance to integrated risk assessment procedures by carrying out a full hazard and risk assessment for four chemicals compound classes:

1. polybrominated diphenylethers,
2. perfluoroalkylated substances,
3. substituted musks/fragrances
4. triazoles/benzotriazoles.

Based on their analysis our project will exemplify the integration of information, models and strategies for carrying out a full ecological safety-, hazard- and risk assessments for large numbers of substances to the new categories of risk assessors within REACH. Real risk estimates will be delivered according to the basic philosophy of REACH of minimizing animal testing, costs, and time. CADASTER will show how to increase the use of non-testing information for regulatory decision whilst meeting the main challenge of quantifying and reducing the level of uncertainty.

During the project we will develop Decision Support System (DSS). It will be updated on a regular basis in order to accommodate newly developed testing strategies, newly generated experimental data, and newly developed predictive models for additional compound classes. The DSS will be a free on-line and also a stand-alone tool for development, publishing and use of QSAR models for REACH. It is aimed to be compatible with the OECD QSAR Application Toolbox and the EPI SuiteTM Toolbox developed by the EPA's Office of Pollution Prevention Toxics and the Syracuse Research Corporation (SRC).

All interesting industrial and academic partners, who are working on these classes of compounds or challenge similar goals, are cordially invited to establish a fruitful collaboration in order to solve common problems together.

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