Abstract of

Poster Presentation

GEOCHEMICAL AND BIOLOGICAL DESCRIPTION OF A CO₂ SEEP SYSTEM IN SHIKINE ISLAND, IZU

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Natural analogues for ocean acidification allow assessments of future trajectories of marine ecosystems. These analogues include volcanic seeps, where CO_2 bubbles up through the sea floor. Not all seeps are well suited to studies of ocean acidification due to anomalies in temperature, salinity, alkalinity and other chemical anomalies. Places are starting to be found, such as Ischia in Italy, that have elevated carbon dioxide levels that are unaffected by confounding factors. The search is now on for replicate CO_2 seep systems to find out how coastal habitats will be affected by rising CO_2 levels.

Here we describe a set of CO_2 seep that were recently discovered around Shikine Island. Geochemical studies show that some of these seeps are suitable for ocean acidification research since the gas contains >98% CO_2 although in some locations there is up to 90 ppm H₂S. Measurements of total sulfide and redox potential has revealed that detectable sulfide levels are limited to a few meters around the seeps due to the re-oxidation of the sulfide to sulfate in the well oxygenated water. Total alkalinity was constant across the high CO_2 gradients and at reference sites around the island. A gradient in pH was observed from pH as low as 6.4 near the venting sites to 8.1 farther away. Areas with geochemistry analogous to that predicted for 2100 were found with no detectable sulfide.

Biological surveys of the intertidal and subtidal zones have shown significant changes in the faunal and algal communities. Calcified animals (scleractinian corals, barnacles, serpilids, molluscs) were highly impacted at high CO₂ levels as were the coralline algae with significantly reduced abundances at mean pH <7.8. nMDS analysis of the faunal and floral community in both the intertidal and subtidal zones showed a strong effect of pH. Our findings are broadly similar to those recorded at seep systems off Papua New Guinea and in the Mediterranean with increases in CO₂ causing drastic changes in sessile community composition. We hope these new results about the effects of rising CO₂ levels on coastal habitats in the NW Pacific will help inform ocean acidification policy in the region..

ELEVATED CO₂ DISRUPTS INVERTEBRATE COLONIZATION ON TROPICAL CORAL REEFS

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Carbon dioxide emissions to the atmosphere are causing ocean acidification; there has been a 30% increase in the concentration of H^+ ions in surface ocean waters since the Industrial Revolution. Levels of Dissolved Inorganic Carbon are continuing to rise rapidly worldwide, causing a fall in the saturation state of carbonate.

Volcanic activity in Milne Bay Province, Papua New Guinea, causes CO_2 gas to bubble up from the seabed through the water column at two shallow (< 5 m depth) coral reefs resulting in localised ocean acidification. These seeps have been active for at least 70 years, creating zones where seawater carbonate chemistry is comparable to that expected as atmospheric CO_2 emissions continue to rise. Work at these sites has already shown dramatic decreases in coral reef biodiversity. Here, we placed settlement pads for one month along pH gradients at the seeps to observe the effects of ocean acidification on marine invertebrate colonization.

We found significant differences in invertebrate colonization between areas of coral reef affected by increased CO_2 levels and adjacent reference sites. Settlement pads from acidified areas were less diverse and had a few resilient taxa that dominated the community. Heavily calcified taxa such as molluscs were severely affected in the low carbonate areas. Within the Crustacea, tanaids were significantly more abundant in the most acidified conditions, whilst amphipods and copepods were significantly less abundant.

Our findings suggest that near-future ocean acidification may reduce the diversity of invertebrates on tropical coral reefs and cause significant shifts in community structure and function, with potentially far-reaching consequences.

THE EFFECT OF GLOBAL WARMING ON PERIPHYTON IN CORAL REEF ECOSYSTEM

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By the end of this century, the expected atmospheric CO₂ concentration will reach 800~1000 ppm. Phytoplankton and benthic algae are the primary production in the ocean, but the effects of CO₂ and temperature on the algae is inconclusive. In this study, we examined the effects of global warming on growth and community assemblages of phytoplankton and benthic algae. The experiments were conducted with two CO₂ treatments (400 ppm and 800 ppm) under two temperatures (28°C and 25°C) in mesocosms. The results indicated that at 28°C, elevated CO₂ enhanced the growth of phytoplankton but reduced the species diversity. Elevated CO₂ increased the benthic algal growth, especially for diatom but not cyanobacteria or dinoflagellates. At 25°C, the relative abundance of cyanobacteria increased while the diatom decreased. CO₂ and temperature alter algal community assemblages, a result that might affect marine primary production and nutrient cycle in the future

POTENTIAL ANTIFOULING NATURAL COMPOUNDS AND THE DEREVITIES AGAINST BARNACLES

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Barnacles are one of the major fouling organisms that can gregariously attach on underwater artificial surfaces. Huge efforts have been made to inhibit or remove the attachment, thusmitigateeconomical losses. The once popular use of organotin in anti-fouling coatingswas proven effective yet causing long-term and lethal damage on marine ecosystems. In the past few decades, development of non-toxic and environmental friendly coatings has been a critical issue yet challenging. In this study, we screened for potential anti-fouling compounds extracted or synthesized from natural products of marine organisms. The efficiency and toxicity of these compounds were evaluated with the 50% effective concentration (EC_{50}) and 50% lethal concentration (LC_{50}) against the cyprid larvae of barnacle *Amphibalanus amphitrite* (=*Balanus amphitrite*). Our preliminary results showed one natural compound S28has lowEC₅₀ as 8.8 ppm and high LC_{50} as 101.1 ppm. This high therapeutic ratio ($TR=LC_{50}/EC_{50}=11.5$) suggested potential application of this compound in future development of anti-fouling coatings. Further studies will be made to investigate the underlying mechanisms at molecular level.

EFFECTS OF FLOODING ON PELAGIC ECOSYSTEM OF THE EAST CHINA SEA

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This study was designed to determine the effects of flooding on pelagic ecosystem in the East China Sea (ECS), especially on plankton community respiration (CR). In July 2010, a flood occurred in the Changjiang River. As a comparison, a variety of both abiotic and biotic parameters were monitored, as well as in July 2009, a non-flooding period. During the flooding, the Changjiang diluted water (CDW) zone covered almost two thirds of the ECS, which was approximately six times that of the non-flooding period. The mean nitrate concentration was higher in 2010 (6.2μ M) than in 2009 (2.0 µM). However, during the 2010 flood, the mean values of Chl a and bacterial biomass were only slightly higher or even lower than in 2009. However, the CR was still higher in 2010 than in 2009, with mean values of 105.6 and 73.2 mg C m⁻³ d⁻¹, respectively. The higher CR in 2010 could be attributed to vigorous plankton metabolic activities, especially phytoplankton, at stations in the CDW zone, which were not characterized by low SSS in 2009. In addition, zooplankton might be another important component contributing to the high CR rate observed in 2010. Furthermore, there was a significant amount of fCO₂ drawdown in the 2010 flood. These results suggest that the flood in 2010 had a significant effect on the carbon balance in the ECS. This effect might become more pronounced in the future, as extreme rainfall events and flooding magnitudes are predicted to increase globally due to climate change.

OCEAN ACIDIFICATION ALLEVIATES MERCURY TOXICITY TO *TIGRIOPUS JAPONICAS* UNDER MULTIGENERATIONAL EXPOSURE SCENARIO

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Ocean acidification (OA) and mercury (Hg) pollution are frequently concurrent problems in marine and coastal environments. However, little information has been provided about the impact of OA on Hg toxicity in marine organisms. Here, we investigated the combined effects of OA and Hg pollution (ambient condition: pCO₂ 400 ppm×control; and stress conditions: pCO₂ 400 ppm×10 µg/L Hg, pCO₂ 1000 ppm× control, pCO₂ 1000 ppm×10 µg/L Hg) on development and reproduction of marine copepod Tigriopus japonicus under multigenerational exposure of four generations (F0-F3). Metal accumulation, as well as six important life history traits, i.e., survival, developmental time for nauplius phase, developmental time to maturation, fecundity, number of clutches, and number of nauplii/clutch was analyzed for each generation. The results indicated that metal accumulation were significantly decreased under the combined exposure (OA and Hg) when compared with that under Hg exposure, furthermore, with a trend for higher Hg accumulation from F0 to F3. Under most circumstances, OA exposure displayed negligible effect on the six traits in the copepod. However, number of clutches, and number of nauplii/clutch was evidently suppressed by Hg treatment. Interestingly, OA could alleviate the Hg inhibitory impacts on number of clutches, and number of nauplii/clutch, and it was attributable to less metal accumulation under the combined exposure than that under the single Hg treatment. Additionally, the combined exposure shortened the development time in contrast to the single Hg exposure. Overall, OA could alleviate the toxicity to marine copepods caused by Hg pollution, and environmental risk assessment of Hg pollution must therefore integrate the elements in global climate change (e.g., OA) so as to provide a realistic measurement of the influences on aquatic ecosystem.

THE COMBINED EFFECTS OF OCEAN ACIDIFICATION AND FOOD AVAILABILITY ON DEVELOPMENT, REPRODUCTION AND SOD ACTIVITY OF *TIGRIOPUS JAPONICUS*

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Anthropogenic emissions of carbon dioxide caused ocean acidification. Some studies have indicated that ocean acidification had little effect on adults of copepod Tigriopus japonicus, but early development of larvae and reproductive capacity of females could be impaired because of higher maintenance costs under high pCO₂-environments. In this study T. japonicus was exposed to natural seawater (pH8.1 as control) and CO₂-induced acidified seawater (pH7.3) under eight food concentrations (5×10³ind/mL, 5×10⁴ind/mL, 1×10⁵ind/mL, 2×10⁵ind/mL, 3×10⁵ind/mL, 4×10^{5} ind/mL, 5×10^{5} ind/mL, 8×10^{5} ind/mL) for two generations to investigate the combined effects of ocean acidification and food availability on development, reproduction and SOD activity of T. *japonicus*. Under both pH levels the optimum food concentration was 2×10⁵ind/mL for larvae development and 3×10⁵ ind/mL for female reproduction. For both natural and acidified conditions, shortage of food supply had significantly negative effects on larvae development and female reproduction, the development time of nauplii and copepodites were prolonged and the nauplius production decreased with the food concentration decreasing. Excess food supply also significantly decreased the nauplius production with the food concentration increasing at both pH levels. Compared to natural condition, seawater acidification significantly retarded the nauplius development under all the food concentrations, while it prolonged the copepodite development time only when the food was in short supply. Seawater acidification also enhanced the negative effect on female reproduction when the food was excess, the nauplius productions at pH7.3 was significantly lower than those at pH8.1. SOD activity of F1 female copepods was significantly higher at pH 7.3 than at pH8.1 under all the food concentrations. At pH 7.3 SOD activity increased with the food concentration decreasing, but at pH8.1 it only significantly higher when the concentration of food was 1×10^5 ind/mL or less. The present study indicated that ocean acidification could enhance the negatively effects on the development and reproduction of T. japonicas when the food supply deviated from the normal needs. SOD activity could be improved to resist the joint threat from ocean acidification and food shortage.

EFFECTS OF SEAWATER ACIDIFICATION ON INTERTIDAL NEMATODES' COMMUNITY STRUCTURE AND DIVERSITY

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Since the start of the industrial revolution, anthropogenic activities have led to a significant increase in carbon dioxide (CO₂) emissions. Ocean is one of the most important sink of this increased CO₂. The oceanic uptake of the increased CO₂ has already caused an average decrease in the global surface water pH by approximately 0.1 units since the preindustrial era. The goal of present study was to investigate potential effects of seawater acidification on intertidal nematodes community structure and diversity by using a microcosm experiment. Nematodes communities for microcosm experiment were collected from the low tidal zone of two beaches in Qingdao (China) in August 2013. Perspex cores of sediment (inner diameter 4.4 cm and 3.4 cm) were separately collected to a depth of 20 cm from sandy sediment in the Yangkou Beach and a depth of 10 cm from the silty sand sediment in the Tangdaowan Beach, and incubated in laboratory microcosms. In microcosm, nematode communities were exposed for 56 days to ten experimental treatments (three replicates for each treatment) comprising two pH levels: 8.0 (ambient control) and 7.3, crossed with five exposure days: 0 day control, 7 days, 14days, 28 days and 56 days. Present study showed that nematode community structure and diversity changed continuously when they are exposed to pH 7.3 seawater. In sandy sediments, abundance of female and juveniles decreased firstly after 7 days exposure. Diversity index H' increased after 14 days exposure. Nematodes of order Monhysterida outweighed that of order Enoplida after 28 days exposure. The feeding structure of nematodes also changed after 28 days. Non-selective deposit feeders (1B) were the most dominant type from 28 days while epistratum feeders (2A) was most dominant before that. It is reported that benthic algae would be restricted by increase of seawater pCO₂. So, the decline of 2A feeders might be induced by decrease of benthic algae which was one of the major food sources for 2A feeders. After 56 days exposure, nematode community structure and dominant species changed significantly. In silty sand sediments, influence of acidification on nematode community structure emerged earlier than in sandy sediments. After 7 days exposure, Monhysterida replaced Chromadorida as the most dominant order. At the same time, 1B feeders dominated in nematode community after 7 days exposure. Diversity index H' also decreased after 7 days. After 56 days exposure, nematode community structure and dominant species also changed significantly.

GROWTH AND TOXICITY OF MARINE BENTHIC DINOFLAGELLATES UNDER THE COMBINED EFFECTS OF TEMPERATURE AND OCEAN ACIDIFICATION <u>J C H Ip</u>¹, S K F Yiu¹, V T T Lam¹, M Yan^{1,2}, T C Wai¹, L L Chan^{1,2}, P K S Lam^{1,2,3} and P T Y Leung^{1,2}

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Marine benthic dinoflagellates can produce toxic secondary metabolites which are harmful to the health of human and marine ecosystem. Some dinoflagellates may benefit from nutrient-rich land runoff, warmer seawater and coral reef disturbance, and become more successful, while others may subside in areas under impact. Currently, there are significant knowledge gaps on how various environmental stresses and their combined effect that alter the growth and the toxicity of the benthic dinoflagellates, in particular the potential exacerbation of their risks to health of humans and marine ecosystems under the warming of seawater and ocean acidification. Recently, a diverse species of the benthic dinoflagellates belonging to the common toxic genera, i.e., Amphidinium, Coolia, Fukuyoa (globular Gambierdiscus), Ostreopsis and Prorocentrum, have been reported in Hong Kong's reef systems. Their potential risks to our local marine environment are however virtually unknown. In this study, we aim to investigate the growth performance and toxicity of the locally collected benthic dinoflagellates under the effects of different water temperatures, pH and their combined. The toxicity of the treated algae will be further examined using bioassays with marine invertebrate larvae, such as the nauplii of the brine shrimp Artemia sp. and the marine copepod *Tigriopus japonicus.* The findings of this study will advance our understanding on risks of these local benthic dinoflagellates to Hong Kong's marine environments, and are helpful to provide essential background information on how these algae respond to the climate change scenarios, in terms of growth and ecotoxicity. Future molecular investigations would warrant the elucidation of the underlying mechanisms of the toxic effects caused by this group of marine dinoflagellates.

Keywords: Benthic dinoflagellate, Climate change, Temperature, Ocean acidification, , Ecotoxicity

EVOLUTION AND EXPRESSION OF THE METAL RESPONSE IN THE ASIAN LANCELET, *BRANCHIOSTOMA BELCHERI*

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<u>BACKGROUND</u>. Lancelets (*Branchiostoma* spp.) are basal chordates and inhabit shallow, coastal regions that are susceptible to ocean warming, hypoxia and pollution. MTF1 (metal response transcription factor 1) has been shown to mediate the induction of metallothionein (Mth) and zinc transporters in response to trace metals. Mth may also be induced in response to hypoxia, possibly as an indirect effect of hypoxia-inducible factor (HIF) on MTF1 activity.

<u>STUDIES</u>. First, at the genomic level, we analysed the evolution of MTF1 in three lancelet species. We also compared the conserved sequence elements in the promoter regions of MTF1 and Mth orthologs in the Asian and Florida lancelet (*B. belcheri* and *B. floridae*), oyster (*Crassostrea gigas*) and zebrafish (*Danio rerio*). Second, at the transcriptional level, we determined the effects of exposure to low levels of cadmium (Cd), nickel (Ni) and chromium (Cr) on the mRNA expression of MTF1 and Mth in *B. belcheri*. We also measured changes in the expression of HIF and two zinc transporters in Cd-treated animals. Third, at the level of the proteome, we employed immobilized metal affinity chromatography (IMAC) followed by 1- gel electrophoresis and silver staining to visualize changes in the abundance of metal-binding proteins ('metallome') in animals treated with Cd or Ni.

<u>RESULTS</u>. (i) In *B. belcheri*, *B. lanceolatum* and *B. floridae* the MTF1 gene encodes a protein with six C_2H_2 -type zinc fingers, the same domain architecture that is found in vertebrates. Bayesian phylogenetic inference confirmed the basal position of cephalochordates in the chordate lineage and indicated that the MTF1 gene in *B. belcheri* diverged earlier from a common lancelet ancestor than did the homologous genes in *B. lanceolatum* and *B. floridae*. (ii) In animals exposed for 72h to 100 ppb Cd, Ni or Cr, Cd and Ni significantly elevated the ratio of Mth mRNA/actin mRNA (35-fold and 24-fold, respectively, p<0.05). MTF1 transcript levels themselves were also increased by Cd and Ni (75-fold and 22-fold, p<0.005). Chromium ion, which has a much larger hydrated radius, was without effect. The order of activity of these metals, Cd > Ni > Cr = Control, correlated with the predicted ability of their hydrated ions to compete for a zinc ion-selective binding site (p<0.01). Modest, but significant increases were also observed in the relative expression of genes encoding HIF and a ZIP14-like zinc transporter (4.7-fold and 8.7-fold) but not a Znt7-like transporter. (iii) Using a cobalt resin, we extracted high-affinity metal-binding proteins from lysates corresponding to equal masses of tissue. Following exposure to Cd or Ni, the relative abundance of high-affinity metal-binding polypeptides was Cd > Ni > Control.

<u>DISCUSSION</u>. The induction by trace metals of Mth, and of MTF1 itself, has been reported previously in zebrafish cells (Cheuk et al., 2008). The 5'-UTR of the zebrafish MTF1 gene lacks a conserved metal response element (MRE) while in the lancelet MTF1 gene, only a single MRE was found 267 nt upstream of 'Start'. These data are consistent with metal-dependent induction of Mth by MTF1, accompanied by an increase in the stability of MTF1 mRNA. Mth can also be induced by hypoxia, so it was interesting to observe a reciprocal effect of metal exposure on the expression of HIF. Expression of a ZIP14-like (inward) transporter was also elevated by Cd, suggesting a feedback response to zinc depletion.

THE RELATION BETWEEN DISSOLVED COPPER, ZINC, COBALT, VANADIUM, IRON AND PHOSPHATE IN THE COASTAL WATERS OF THE EAST CHINA SEA <u>H Su</u>, R J Yang, A B Zhang, Y Li and X C Wang

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The spatial distribution of dissolved copper, zinc, cobalt, vanadium during autumn survey from October 21 to November 2, 2011 in the East China Sea (ECS), were analyzed using inductively coupled plasma mass spectrometry (ICP-MS). These trace metal in the ECS can be divided into two groups: nutrient like group (Cu and Zn) and non-nutrient like group (Co and V). Our correlation analysis results between trace metals and nutrients (by Statistical Product and Service Solutions software) shown that total dissolved Cu and Zn negatively correlated with total dissolved phosphate (TDP). The pearson correlation coefficient between Cu, Zn and TDP (P), were -0.272 and -0.275, respectively, while dissolved Co and V didn't correlate with nutrients. At the same time, in order to understand the absorption of trace metal and phosphate by marine plankton, dissolved iron and its organic speciation were investigated by competitive ligand equilibration-adsorptive cathodic stripping voltammetry (CLE-ACSV). Our results indicated that the dissolved Fe significant negatively correlated with its organic ligand (Lt, P, 0.986), and the inorganic Fe significant negatively correlated with TDP (P, -0.420). These correlations suggested that there were the tight coupling between those nutrient-like trace metals with nutrient elements and phytoplankton, which were due to the limiting phosphate in the East China Sea. The average concentrations of dissolved Cu, Zn, Co, V were 41.80±5.56 (average ± standard deviation), 22.59±8.92, 0.94±0.18, 20.36±5.58 nmol/L, respectively. Dissolved Fe concentrations ranged from 4.30 to 82.35 nmol/L averaging 16.22±16.62 nmol/L, and Lt from 4.88 to 89.30 nmol/L averaging 19.61±18.11 nmol/L. The highest values of dissolved Cu, Zn, Co, V and Fe were all observed in Yangtze River estuary and the bottom waters, due to the input of Yangtze River water and the release from bottom sediment. Furthermore, Yellow Sea Warm Current caused the disturbance of the sea water, which resulted in the releasing of sediment from bottom and increased concentration of the dissolved trace metals.

BIOCONCENTRATION, METABOLISM AND BIOMARKER RESPONSES IN MARINE MEDAKA (*ORYZIAS MELASTIGMA*) EXPOSED TO SULFAMETHAZINE

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To investigate distribution, bioconcentration, metabolism, and biomarker responses of sulfonamides antibiotic sulfamethazine (SM₂) and its main metabolite acetylationin sulfamethazine (N-SM₂) in fish, medaka (Oryzias melastigma) were exposed to 40 and 200 μ g L⁻¹ of SM₂ for 24 h to estimate their potential environmental emergency risk. The SM₂ content in different tissues was quantified by UPLC/MS/MS. The bile exhibited the highest SM₂ concentration followed by the liver, gonad, gills, and muscle tissues in male fish, while liver showed the highest level in female fish; as to the acetylationin metabolite, it showed a different distribution from that of parent compound, the main enriched organ was gonad except for low dose in female fish. In addition, biomarkers, including superoxide and catalase dismutase in the liver, had changed significantly after 2, 12, and 24 h of exposure (P < 0.05), and presented a double-peak phenomenon. These results suggested that SM₂ could be absorbed and metabolized through multiple routes by fish in short time and N-SM₂ was mostly accumulated in gonad tissue. Therefore, interactions between SM₂ or its metabolites and biological systems may induce biochemical disturbances in fish, and further study will be needed to probe into the related toxicity mechanism.

POLLUTION OF COASTAL STREAMS FROM AGRICULTURAL ACTIVITIES: ALEXANDER RIVER CASE STUDY

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The riverine system is a known receptacle for diffusive pollutants. These pollutants, with an emphasis on pesticides, are often originated from cultivated fields and enter the aquatic environment mainly through leaching, spray drift, surface run-off and soil erosion. The latter refers mainly to soil particles, detached due to the kinetic energy transferred from raindrop impact on bare soil, and transported with run-off from the fields to the riverine system. These particles have the potential to travel vast distances in suspension and settle downstream in depositional areas such as estuaries and the continental shelf. The transported and settled particles carry the potential of containing adsorbed pesticides which may affect the aquatic environment.

The pollution potential of the Alexander estuary (560 km²) from cultivated fields was examined. Seven flood events were sampled during the rainy seasons of 2013/14 and 2014/15. A screening of over 250 pesticides and metabolites was conducted to the flood solution and suspended sediments separately. Overall, 65 pesticides were detected of which 39 were quantified with peak concentrations of 23 µg/l and 5.5 mg/kg of dissolved and adsorbed fungicide (Tebuconazole) respectively. Suspended sediments bound pesticides consisted between 10 to 20 percent of the total pesticides flux. This fraction importance is not restricted to its amount contribution alone yet to its environmental behaviour as well, Such as in the Alexander estuary case which often acts as a sink for particulate matter. Comparing to a feasibility screening of the river's base flow during summer and spring (dry season), it appears that the contribution of flood events to the total annual load of pesticides submerges that of the rivers base flow which consist mostly of reclaimed water.

The agricultural activities in the region are intensifying over time and so is the use of pesticides. Combined with higher intensity rain storms, due to climate change, soil degradation is a major concern along with its impact on the surrounding environments. Suspended sediments bound organic pollutants, emitted from the cultivated fields, flows directly to the Alexander estuary and Mediterranean sea and in order to understand their fate in these environments additional studies are required.

BASELINE CONCENTRATIONS OF SELENIUM IN MUSCLE, KIDNEY AND LIVER TISSUES OF FOUR SMALL TAIWANESE CETACEANS IN THE WESTERN PACIFIC OCEAN

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Selenium, silver and cadmium concentrations in the muscle, kidney and liver tissues of 33 bycatch and stranded cetaceans, including 12 *Grampus griseus* (*Gg*), 5 *Kogia simus*, 7 *Lagenodelphis hosei* (*Lh*), and 9 *Stenella atteunuata*, were analyzed. The samples were collected in waters off Taiwan from 1994 to 2012. By Multiple Dimension Scale analysis of the data, the concentrations (in μ g/g dw) of Se in the cetaceans were established as muscle (2.66±1.01) < liver (13.60±5.99) = kidney (16.81±8.32) as the baseline, and muscle (11.54±11.64) < kidney (30.19±11.40) < liver (259.81±150.20) as the unhealthy level. The major contributions to separating the two groups were liver-Se (38.30%) and kidney-Cd (22.37%). Liver-Se exceeding 260 µg/g dw can be a good indicator of heavy metal poisoning in cetaceans. Lower Se concentrations found in the Taiwanese cetaceans indicated their weaker detoxification ability.

EFFECTS OF STABLE AQUEOUS FULLERENE NANOCRYSTAL ON *DAPHNIA MAGNA*: LINKING BIOMARKERS TO BEHAVIORS

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Fullerene (C_{60}) has been employed in a great many applications including batteries, fuel cells, and composites with a sharply increasing production. However, there is a increasing concern about the potential hazards of C₆₀ to the ecosystem and organisms. The main objective of this paper is to investigate the toxic effects of C_{60} on *Daphnia magna*. The results revealed that the adverse effects of C_{60} on daphnids were not obvious at a concentration of 1 mg/L. In contrast, higher C_{60} concentrations led to significant immobilization and increasing mortality of daphnids. The EC₅₀ values of C₆₀ at 48h and 72h were 25.3 \pm 1.5 mg/L and 14.9 \pm 1.2 mg/L, respectively. A dose-depen Fullerene (C₆₀) has been employed in a great many applications including batteries, fuel cells, and composites with a sharply increasing production. However, there is a increasing concern about the potential hazards of C₆₀ to the ecosystem and organisms. The main objective of this paper is to investigate the toxic effects of C_{60} on Daphnia magna. The results revealed that the adverse effects of C₆₀ on daphnids were not obvious at a concentration of 1mg/L. In contrast, higher C₆₀ concentrations led to significant immobilization and increasing mortality of daphnids. The EC₅₀ values of C₆₀ at 48h and 72h were 25.3±1.5 mg/L and 14.9±1.2 mg/L, respectively. A dosedependent decrease in survival was observed for D. magna with a 48h LC $_{50}$ value of 28.5 \pm 1.9mg/L and a 72h LC₅₀ value of 16.3 ±0.8mg/L. In addition, the daphnids exhibited behavior changes under the exposure to C_{60} , which was supported by the increasing of hops and heartbeat frequency within the first few hours, but a decreasing with prolonged exposure. Furthmore, the decreasing activity of the enzyme acetylcholinesterase (AChE) and an obvious oxidation stress were observed after 72h exposure of C₆₀ at concentrations \geq 5 mg/L, suggesting that a relationship might exist between the behaviors of D. magna and its biomarkers. This research can be helpful to the further study of toxicological impacts of C₆₀ on the aquatic organisms.

METAL CONCENTRATIONS IN THE WHITE MUSCLE OF BIGEYE TUNA (*THUNNUS OBESUS*) FROM THE ATLANTIC AND INDIAN OCEANS

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Six heavy metals (As, Cd, Cu, Fe, Se, Zn) in the white muscle of bigeye tuna, *Thunnus obesus*, from the Atlantic (n=53) and Indian (n=34) Oceans were analyzed. Significantly higher As muscle concentrations were found in the Indian Ocean than in the Atlantic Ocean tuna, but no significant regional differences were found in the other metals. Positive correlations between Cd muscle concentrations and fork length (FL, cm) in tuna found in both oceans, and Fe muscle concentration and FL in the Atlantic tuna were established. The female Cu muscle concentrations were higher than those of males in the Indian Ocean. High Se-HBV revealed an antagonism between Se and Hg. Only 10.3% of the bigeye tuna larger than 145 cm FL from the two oceans contained Cd muscle concentrations exceeding the food safety limit (0.1 μ g/g w.w.) set by the European Commission (EC, 2006) and the European Food Safety Authority (EFSA, 2007).

SILVER AND CADMIUM CONCENTRATIONS IN THE TISSUES OF FOUR SMALL TAIWANESE CETACEANS INDICATING THE Ag AND Cd POLLUTION IN THE WESTERN PACIFIC OCEAN

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Muscle, lung, kidney and liver tissues of 45 bycatch and stranded cetaceans, including 14 *Grampus* griseus (*Gg*), 7 *Kogia simus*, 10 *Lagenodelphis hosei* (*Lh*), and 14 *Stenella atteunuat*a, were collected in waters off Taiwan from 1994 to 1995, and from 2001 to 2012. Baseline concentrations (in $\mu g/g \, dw$) of the cetaceans were lung (<0.05) = muscle (<0.05) < kidney (0.08±0.04) < liver (0.55±0.50) for Ag, and muscle (0.05±0.04) = lung (0.24±0.20) < liver (5.03±4.43) < kidney (15.56±16.88) for Cd. Unhealthy Ag and Cd bioaccumulative concentrations of the tissues in the small cetaceans are suggested. Marked high concentrations of Ag and Cd found in *Gg* and *Lh* seem to be highly related to their squid-eating habits. Highest recorded concentrations of liver-Ag and kidney-Cd were found in two *L*h. These Taiwanese cetaceans indicate marked Ag and Cd pollution in the recent two decades in the western Pacific Ocean.

IMPACTS OF CADMIUM EXPOSURE ON LIFE HISTORY TRAITS OF *TIGRIOPUS JAPONICUS* UNDER MULTIGENERATIONAL EXPOSURE AND SUBSEQUENT RECOVERY FROM POLLUTION

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Tigriopus japonicus is a good model organism for marine ecotoxicology. In this study, T. japonicus were exposed to different concentrations of cadmium (Cd) in seawater (nominal concentrations of 0, 2.5, 5, 10, and 50 μ g/L) for five consecutive generations (F₀-F₄), and it was followed by subsequent recovery of two generations (F5-F6) in clean seawater. Metal accumulation and six important life history traits (i.e., survival, development time for nauplius phase, development time to maturation, fecundity, number of nauplii/clutch, number of clutches) were examined for each generation. The results showed that Cd accumulated in the treated copepods with a concentration dependent manner during F_0 - F_4 , and it showed a trend of higher metal accumulation with increasing generations. However, during recovery of F₅-F₆, Cd slightly accumulated in the treated copepods, especially, all the treated metal contents displaying little difference from the control on in F₆. Under multigenerational exposure (F_0 - F_4), development time for nauplius phase, development time to maturation, fecundity, and number of nauplii/clutch showed a sensitivity to Cd toxicity, and the effects tended to worsen from F₀ to F₄, highlighting that cumulative effects were involved into Cd multigenerational toxicity and it was attributable to higher metal accumulation with increasing generations. Interestingly, during recovery, the four sensitive traits in F₅ were also affected by the pre-multigenerational Cd exposure, although it exerted little influences in F₆, suggesting a maternal effect from the multigenerational exposure. Overall, environmental risk assessment of Cd pollution must therefore consider the multigenerational exposure and its maternal effects so as to provide a realistic measurement of the impacts on aquatic ecosystem.

ENVIRONMENTAL AMMONIA INDUCES THE RESPONSES OF ACUTE PHASE RESPONSE POTENTIALLY MEDIATED BY ENDOPLASMIC RETICULUM STRESS IN ASIAN SEABASS (*LATES CALCARIFER*)

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Ammonia is a common pollutant and elevated environmental ammonia occurs facilely in aquaculture ponds with intensive recirculating aquaculture systems to cause immune disturbances in aquatic animals. However, the impacts of waterborne ammonia on acute phase response (APR), a core innate immune response, in aquaculture species is not well understood. The present study explored the molecular responses of genes encoding acute phase proteins (APPs) (i.e., SAA, SAP, CRP, HAP LATE1, LATE2 and IL1 β) to sublethal ammonia exposure (0.2 and 0.4 mg/L total ammonia-N) in liver of Asian seabass (*Lates calcarifer*). After 4-day exposure, upregulation of SAA, LATE1, LATE2 and HAP was found at 0.4 mg/L ammonia exposure. Furthermore, an increase in transcriptional response of APPs was probably through ammonia-induced endoplasmic reticulum (ER) stress supported by elevation of unfolded protein response (UPR) genes (BiP, CHOP and GRP94) and proteins (BiP and GRP94). This study is the first time to report the potential regulatory mechanism that sublethal ammonia exposure induces ER stress may mediate activation of acute phase response in fish.

EFFECT OF HYDROTHERMAL DISCHARGE ON SURFACE DISTRIBUTION OF METALS IN COASTAL SEDIMENTS OFF NORTHEASTERN TAIWAN

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Submarine hydrothermal vents located mainly on eastern sites off Kueishantao Islet (KST) discharge acidic fluid containing high concentrations of sulfide, CO₂(g) and trace metals. The KST located about 10 km away from northeast of Taiwan. The discharged fluid may affect the distribution of trace metals in surface sediments near or away from hydrothermal vents, as the venting fluid mixed quickly with the surrounding seawater. In addition to total concentration of trace metals (Al, Fe, Mn, Cu, Zn, Pb, Cd, Co, Ni, As) in sediments, several indicators of hydrothermal emission such as the concentrations of total sulfide, leachable metals, concentration ratios of [A1]/[A1+Fe+Mn] and [Fe]/[Mn], and geochemical enrichment factor in sediments were used for tracing the effect of discharged fluid on surface sediments. Total concentrations of metals did not show the highest near hydrothermal vents where the sediments contained less contents of mud and organic carbon. However, the fraction of leachable metal was likely elevated in sites influenced by discharged fluid. The concentration of total sulfide, geochemical enrichment factor and [Fe]/[Mn] decrease generally as the distance increases away from the vents. Whereas the [Al]/[Al+Fe+Mn] ratio increases as the distance increases away from the vents. Principal component analysis was also applied to classify the locations and geochemical parameters to reveal the effect of hydrothermal discharge on metal distributions in the studied area.

CHLORINE DIOXIDE AS AN ALTERNATIVE ANTIFOULING BIOCIDE FOR COOLING WATER SYSTEMS: TOXICITY TO THE LARVAL BARNACLE AMPHIBALANUS RETICULATUS (UTINOMI)

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Conventionally, chlorine has been the biocide of choice for biofouling control in large oncethrough seawater cooling systems. However, problems such as reduced biocidal activity at high seawater pH, formation of toxic disinfection by-products and consumption by interaction with organics have necessitated the search for an alternate biocide. Chlorine dioxide (ClO₂), which is a strong oxidant and relatively non-reactive to organics, seems to be an effective alternative biocide. In this study, acute toxicity of chlorine dioxide to larval forms of the fouling barnacle Amphibalanus reticulatus was investigated to arrive at an effective dose required for industrial application. Increased exposure times to ClO₂ affected the survival and delayed the metamorphosis of naupliar stages (II, IV and VI) of the barnacle. ClO₂ was found to be effective at low residual of 0.1 mgL⁻¹; brief exposures for 20 min elicited 45-63% reduction in naupliar metamorphosis, 70% inhibition of cyprid settlement and 80% inhibition of metamorphosis to juveniles. Increase in concentration (0.2 mgL⁻¹) did not result in significant difference in settlement (78%) and metamorphosis (77%). Further increase in concentration to 0.5 mgL⁻¹ elicited only a marginal increase in settlement and metamorphic inhibition (86 and 74%, respectively). This was partly attributed to mortality of the exposed cyprid larva. Our earlier studies with Cl₂ revealed that in order to elicit appreciable inhibition in barnacle settlement, concentrations greater than 0.5 mgL⁻¹ need to be employed; however, use of such high concentrations would not be environmentally acceptable. In comparison, 0.2 mgL⁻¹ of ClO₂ was able to elicit substantial reduction in the settlement of barnacle larvae compared to control. The study indicates the possibility of using ClO₂ as an alternative antifouling biocide in power plant cooling water systems. However, more work will be required on the environmental effects of such switchover.

Key words: Balanus reticulatus, biofouling control, barnacle, chlorine dioxide, larval

DUAL STRESSOR OF NODAVIRUS INFECTION AND TRIBUTYLTIN EXPOSURE INDUCES HIGHER MORTALITY THAN THE SINGLE STRESSOR IN MEDAKA ORYZIAS LATIPES LARVAE

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The toxic effects of tributyltin (TBT) on fish immunity at the leukocyte level have been well reported, however, the relationship between TBT exposure and occurrence of fish diseases remains unclear. In this study the effect of dual stressor of nodavirus infection and TBT exposure was investigated in medaka, *Oryzias latipes*.

As preliminary experiments, we calculated a 50 % lethal dose (LD₅₀) of the virus and a 50 % lethal concentration (LC₅₀) of TBT for medaka larvae (day 0 after hatching), and they were $10^{4.5}$ tissue culture infectious dose (TCID)₅₀/ml and 46.7µg/L, respectively. In this study, two groups of medaka larvae were prepared. One group was infected with the nodavirus at the titer of $10^{2.5}$, $10^{3.5}$ or $10^{4.5}$ TCID₅₀/ml by immersion for 96 hours. Another group was used as control. After 96 hours, each group was divided into two subgroups for TBT exposure at the concentration of 0, 0.17, 0.52, 1.56 or 4.67 µg/L. Mortality was monitored for 192 hours. Virus titer in the fish was measured using the E-11 cell line and changes in expression of antiviral activity related genes, including interferon γ inducible protein 30, granzyme and perforin were evaluated by real-time PCR.

Of the twelve dual stressor groups, six groups showed significantly higher mortalities than the single stressor groups and control group. Mortality was $45.0\pm5.0\%$ in a dual stressor group received $10^{2.5}$ TCID₅₀/ml of the virus and 0.52 µg/L of TBT that was a combination of the lowest observed effective dose and concentration (LOED and LOEC) among the six groups. These results suggest that the dual stressor induces high mortality in the fish, even when each single stressor had no or low effect. The virus titer in the dual stressor group exposed to the virus and TBT at their LOED and LOEC was higher than that of virus control group, suggesting that TBT exposure accelerated virus replication in the dual stressor group. Moreover, the expressions of antiviral activity related genes in the virus infection and the dual stressor groups significantly increased compared with those of TBT control and solvent control groups. However, the dual stressor group showed lower induction tendency in the genes than the virus infection group. These combined results between virus titers and changes in gene expression suggest that the dual stressor suppresses antiviral activities of the fish and induces high replication of the virus, resulting in high mortality in the fish.

BRAIN SIZE REDUCTION AND ABNORMAL SWIMMING BEHABIOR CAUSED BY PYRENE EXPOSURE IS EVOKED BY AHR INDEPENDENT MECHANISM

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Spills of heavy oil (HO) over the oceans have an adverse effect on marine life. In the previous study, we demonstrated that fertilized eggs of pufferfish (Takifugu niphobles) exposed to HO could induce nervous system disruption and swimming abnormality in early-hatched larvae. We also fond that pyrene, a member of Polycyclic Aromatic Hydrocarbons (PAHs), contribute to the abnormality in the size of the brain and the swimming behavior. However, which signalling pathway dominate a series of toxicological effects has been poorly understood. In the present study, to identify whether Aryl Hydrocarbon Receptor (AHR), which is thought to be a downstream target of PAHs, is involved in the toxic effects caused by pyrene, we performed exposure experiments using β -naphthoflavone, an agonist of AHR, in the following steps: fertilized eggs at 24 hours post-fertilization were exposed to seawater of 25 µg/L, 50 μ g/L, 80 μ g/L and 100 μ g/L pyrene, and 0.1 μ g/L, 1 μ g/L, 10 μ g/L and 100 μ g/L β -naphthoflavone at water temperature 17°C for 8 days, and then they were transferred to fresh seawater before hatching. For solvent control experiments, we used seawater including 0.01% acetone. At two days post-hatching, their swimming behavior, body morphology and size of the central nervous system (CNS) were observed. We found abnormal swimming behavior and reduced sizes of eyes and the midbrain in the larvae exposed to 80-100 µg/L pyrene, as observed in the previous study. However, we could not find those problems in 25-100 μg/L pyrene and 0.1-10 μg/L β-naphthoflavone exposed larvae, although 100 μg/L βnaphthoflavone exposed larvae displayed severe abnormality in the whole body morphology. On the other hand, we found the reduction of heart-beat rate and enlarged volume of the yolk in the 1-100 µg/L and 10-100μg/L β-naphthoflavone exposed larvae, respectively, while pyrene exposed larvae represented no abnormality in the heart-beat rate and size of the york. Thus, it is suggesting that toxic effects of pyrene and β -naphthoflavone to the pufferfish larvae are fundamentally different: pyrene specifically induces problem in the developing CNS and swimming behavior; β-naphthoflavone affects to the function of heart and consumption of the yolk. These difference strongly suggest that the behavioural and morphological abnormality caused by pyrene exposure is mediated by AHR independent pathway.

CDI DESALINATION OF SEAWATER USING ACTIVATED CARBON RECOVERED FROM PALM-SHELL WASTES

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Seawater desalination is of increasing importance mainly because of the shortage of clean water worldwide. However, most of the traditional desalination methods have the major drawbacks of high energy consumption and high operational cost. Palm-shell wastes were used as the carbon source for preparation of activated carbon (AC) for capacitive deionization (CDI) electrodes. The palm-shell AC have a very high surface area of 1200 m³/g with the specific capacitance of electrodes of 185.9 F/g. The CDI efficiency for seawater desalination using the AC electrodes is as high as 60%. Notably, the CDI electrodes can be regenerated effectively. In addition, CDI also has promising applications in the ion removals from contaminated groundwater and industrial waste waters.

EFFECT OF ALUMINUM TOXICITY TO AQUATIC ORGANISMS AND THE CORRELATION BETWEEN THE HARDNESS

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Known as trace elements, Aluminum is very few in the content of organism. But the wide use of Aluminum in industry has led to high vivo residual Aluminum in water, soil and all kinds of aquatic and terrestrial plants and animals, which has a certain influence on the health of organism. In order to have a thorough understanding of the effect characteristics of Aluminum to the aquatic toxicity and the ecological risk in China, it is necessary to conduct a research about the effects of Aluminum toxicity to aquatic organisms. Water hardness has influence on toxicity of Aluminum, but relavent research is relatively few at present. This article takes the freshwater ecosystems as protection target, collect and screen the toxicological data of Aluminum to aquatic organisms, analyze the relationship between hardness and the toxic effect of Aluminum, the results showed that the Aluminum toxicity to aquatic organisms decreased with the increasing of water hardness, the slope is 0.5600. The species sensitivity distribution method was adopted to deduce the criteria to protect freshwater aquatic organisms against Aluminum in China. Through hardness correction, at the hardness of 50 mg·L⁻¹, acute concentration and chronic concentration of Aluminum respectively are 294 $g \cdot L^{-1}$ $g \cdot L^{-1}$. The results could be used to provide the theoretical foundation of ecological risk and 20 assessment and pollution control of this kind of metal.

COASTAL AVIAN RESPONSE TO THE MANAGED DEGRADED MANGROVE WETLAND

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The loss and degradation of mangrove wetland of inner deep bay caused by land-use changes and other anthropogenic activities adversely affected wetland depended species, especially water birds. Maipo Nature Reserve, Hong Kong was originally a mangrove wetland transferred to Geiwei ponds. To mitigate the adverse influences, almost all Geiwei ponds were reconstructed according to preset biodiversity management zones (BMZs) starting from 1990s. This study analyzed if current birds distribution did relate to the BMZs environment. Canonical Correspondence Analysis was performed. The scatter plots based on environment variables of twelve studied Geiwei ponds showed that ponds of the same BMZ generally clumped in the same quadrant or in a short distance on the graph (e.g. pond 3, 4, 7 and 8 clumped in the same quadrant as they all were BMZ of traditional mangrove Geiwei ponds). The above result indicated that a BMZ zone produced common habitat traits. Water bird distribution in twelve studied Geiwei ponds displayed a similar pattern, i.e., ponds in close distance on the CCA scatter plot had similar bird abundance or community composition while long distance ponds had obvious difference (e.g. pond 5 and pond 6, the dominant category was Phalacrocorax carbo and wader, respectively; pond 2, 3, 4, 7, 8 and 12, all had low bird abundance). Although some birds' occupancies missed BMZs expectation, most birds' habitat utilization accorded with manager's BMZ conservation strategy. There was obvious correlation between *P. carbo* and big tree, waders (refer to Charadriidae and Scolopacidae) and bare ground inner ponds and open water. It plausibly indicated that control of key habitat factors accounted for the success of reconstruction and management in Maipo nature reserve.

SCALING PHYTOPLANKTON BLOOM RELATED TO RAINFALL MAGNITUDE IN A HYPER-EUTROPHIC LAGOON: A CONTINUOUS MONITORING SYSTEM APPROACH

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To evaluate how rainfall magnitude effect on phytoplankton bloom, the continuous monitoring systems were deployed and recorded throughout the year of 2015 in a hyper-eutrophic lagoon in southern Taiwan. Results showed that the rainfall was in the range of $0.5 - 129.5 \text{ mm d}^{-1}$, and it occurred frequently during July to August. Corresponding to rainfall, the salinity decreased with rainfall magnitude increasing, and the inverse relationship between salinity and rainfall was significantly evident two days after rainfall ceased. Accompany with rainfall event, phytoplankton bloom was sequentially occurred, and phytoplankton biomass (i.e., Chl *a*) increased significantly with salinity decreasing. Hyper-saturation of dissolved oxygen was also observed during bloom period. The increasing of phytoplankton biomass might befueled by nutrient enriched from drainages located in the inner lagoon. It also implies that the occurrence and magnitude of phytoplankton might become more pronounced in the future, as extreme rainfall events are predicted to increase globally due to climate change.

THE INFLUENCE OF AMBIENT AMMONIA, NITRITE AND COMBINED EFFECT ON PHENOLOXIDASE SYSTEM OF WHITE SHRIMP (*LITOPENAEUS VANNAMEI*)

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Aquatic animals would naturally or anthropogenically face to environmental stress which leads to many physiological disturbances, including interference in immune response. The shrimp Litopenaeus vannamei is an economically important species worldwide. Intensive farming with recirculating system is the common aquaculture practices nowadays that easily result in building up the levels of nitrogenous wastes, especially ammonia and nitrite. Since diseases outbreak would causes major economic loss in aquaculture industry, the immunity is the main sector against pathogen invasion. This study aimed to investigate the potential threat of isolated and combined exposure to ammonia, and nitrite to innate immunity of white shrimp through study on transcriptional and enzymatic responses of phenoloxidase (PO) system in hemocytes. The result revealed that not only total hemocyte count and the expression levels of proPO-activating system related genes, proPO1, proPO2, proPO activating enzyme 1 (PPAE1), PPAE2, prophenoloxidaseactivating factor (PPAF) and serine proteinase (SP), in hemocytes but also PO activity in plasma and hemocytes were significantly decreased in white shrimp exposed to isolated and combined stress of ammonia and nitrite. These findings was suggested that rising waterborne ammonia and nitrite individually or simultaneously may injure the hemocytes leading to decrease in total hemocyte count which subsequently cause disruption of the molecular and enzymatic responses of PO system. Therefore, the susceptibility to pathogen infection of white shrimp might be enhanced. This study explored the interaction between water quality (nitrogenous wastes) and health status of shrimp (innate immunity) to provide the useful information for establishing appropriate criteria for water quality management of aquaculture ponds to avoid severe harmful effects and disease outbreak.

DEVELOPMENT OF PEPTIDE NUCLEIC ACID-DIRECTED PCR CLAMPING FOR INVESTIGAGION OF FEEDING HABIT OF CRUSTACEANS DURING EARLY LIFE STAGES

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Investigation of feeding habit is essential to elucidate inter-specific relationships in coastal ecosystems. However, crustaceans usually crunch prey organisms, resulting in difficulty in identifying prey species by traditional morphological observation of the stomach contents. Recent development in the molecularbased techniques, such as amplification of DNA in stomach contents of a host organism by polymerase chain reaction (PCR) and the following sequencing analysis, may be applicable to investigate feeding habit of crustacean species. However, contamination of a substantial amount of host tissues, especially when analysing larvae or juveniles due to difficulty in separating stomach contents and adjacent host tissues, could result in impaired efficiency for detecting prey DNA sequences. In the present study, we developed a method of PCR-based diet analysis using the peptide nucleic acid (PNA) as a blocking probe for inhibiting DNA amplification of a crustacean host organism, mantis shrimp Oratosquilla oratoria, from Tokyo Bay. We designed universal primers to amplify 28S rDNA for aquatic organisms of a wide range of taxon in Tokyo Bay. We also designed a PNA probe specific to mantis shrimp 28S rDNA. We conducted PCR for DNA template of host (mantis shrimp) and non-host organisms with the universal primers and PNA probe, and confirmed that inhibition of host DNA amplification occurred successfully. Preliminary PNA-based analysis on samples including stomach and adjacent tissues (e.g., carapace and pleopod) of mantis shrimp larvae and juveniles collected from Tokyo Bay detected potential prey organisms, such as copepods and polychaetes. However, we also detected sequences of non-prey organisms, such as ctenophores whose mucus was attached to the external surface of the host organism. Further modification is required to minimize the effect of non-host contamination, and achieve more efficient detection of prey organisms of mantis shrimp.

PHYSIOLOGICAL RESPONSES OF *KANDELIA CANDEL* SEEDLINGS EXPOSED TO MULTIPLE HEAVY METALS

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The contamination of Cu, Pb and Zn of mangrove sediment is heavy in china, expecially in Futian mangrove forest sediment. So it is very necessary to study how Kandelia candel, the major species of Futian mangrove forest, responses to the multiple heavy metals. Treated with multiple heavy metals (Cu, Pb and Zn) for 135d, the dry biomass, water content, root-shoot ratio, relative growth rate, soluble sugar content, malondialdehyde (MDA) and the net photosynthetic rate (P_N), stomatal conductance (g_s), transpiration rate (E) and intercellular CO₂ concentration (C_i) of K.candel seedlings were determined. The results showed that the dry biomass of the leaf, stem and root were comparable among the three treatments during the whole experimental period (p > 0.05). At the beginning (day 15), the water content of the leaf, stem and root was lowest and then kept stable. Under high treatment (400 mg Cu, 400 mg Pb and 600 mg Zn kg⁻¹ dry weight), root-shoot ratio was higher than control and heavy metals could stimulate the growth of the root. Relative growth rate was decrease with the time. At day 15 and day 105, P_N, g_s, E and C_i of high treatment is lower than control. Soluble sugar content and malondialdehyde(MDA) of the leaf both were high at first, then reduced, and increased again. And they were higher than control at the beginning(day 15) and at the end (day 120 and day 135). The present study demonstrates that though heavy metals did have effect on some physiological indexes of K. candel, K. candel still could maintain normal growth and had no significant damage symptoms. And K. candel have a stronger resistance to multiple heavy metals.

IN VIVO EFFECTS OF UV RADIATION ON FATTY ACIDS SYNTHESIS AND EXPRESSION PROFILES OF FATTY ACID SYNTHESIS AND ANTIOXIDANT MECHANISMS IN THE CYCLOID COPEPOD *PARACYCLOPINA NANA*

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In our previous study, we found a slight reduction in the composition of essential fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in the ultraviolet (UV) radiation exposed copepod *Paracyclopina nana* with lipid peroxidation. To evaluate the effects of UV radiation on fatty acid synthesis, *P. nana* was irradiated with several doses (1,2, and 3 kJ/m²) of UV radiation. After UV irradiation, they were incubated for 48 h in dark condition with ¹³C labelled phytoplankton (*Tetraselmis suecica*). Then, the newly produced fatty acids derived from diet were measured using GC-IRMS. After incubation, ¹³C labelled EPA contents was significantly reduced with dose dependent manners. It suggested that the transfer of dietborn EPA was modulated under UV exposure in *P. nana*. The mRNA expressions of fatty acid synthesis related enzymes also modulated in UV exposed copepod. However, there was no significant change in DHA in *P. nana*. Additionally, no DHA contents were measured in the phytoplankton *T. suecica* used as a diet demonstrated that DHA is fully synthesized in copepod after feeding. Thus, we can conclude that DHA synthesis was not influenced by UV irradiation in this study. However, the reduced DHA observed in our previous study indicates DHA, already assimilated in zooplankton was affected by UV irradiation.

ACUTE TOXICITY OF ORGANIC ANTIFOULING BIOCIDES TO PHYTOPLANKTON AND ZOOPLANKTON

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The toxicity of the organic antifouling biocides Irgarol 1051, Diuron, Chlorothalonil, Dichlofluanid, Sea-nine 211, zinc pyrithione, copper pyrithione, Zineb, and Ziram were evaluated against Nitzschia pungens and Artemia sp. Toxicity was calculated as the median effective concentration at 96 h (EC₅₀, 96 h) for *N. pungens*, from the comparative growth rate that was determined by cell counting number. The median lethal concentration at 48 h (LC₅₀, 48 h) for Artemia sp. from the number of dead larvae. The range of concentrations of each antifouling compound was $0-2,000 \ \mu g \ l^{-1}$ for phytoplankton and $0-100 \ m g \ l^{-1}$ for zooplankton. Growth of *N*. *pungens* totally ceased at concentrations $>1,000 \ \mu g \ l^{-1}$ of most of the antifouling biocides tested. In the case of Irgarol 1051, toxicity was extremely high with a low EC₅₀ (0.586 μ g l⁻¹) against N. pungens, whereas the less toxic Dichlofluanid exhibited a high EC₅₀ concentration of 377.101 µg 1⁻¹. The toxicity of antifouling biocides against *N. pungens* was, in order of decreasing potency: Irgarol 1051 > copper pyrithione > Ziram > Zinc pyrithione > Diuron > Zineb > Sea-Nine 211 > Chlorothalonil > Dichlofluanid. In the case of Artemia larvae, no observed effective concentration for larval survival was observed up to 10 mg l^{-1} of Diuron and Zineb. The LC₅₀ of Sea-nine 211 (0.318 mg l⁻¹) and copper pyrithione (0.319 mg l⁻¹) were determined at low levels of these two test biocides, which were the most toxic to Artemia larvae. The overall order of toxicity of antifouling biocides to Artemia larvae was Sea-Nine 211 > copper pyrithione > Chlorothalonil > Zinc pyrithione > Ziram > Irgarol 1051 > Diuron > Zineb > Dichlofluanid. These results provide baseline data concerning the toxicity of frequently used antifouling biocides against marine organisms and in marine ecosystem monitoring and risk assessment.

HEAVY METALS AND ENDOCRINE DISRUPTING CHEMICALS (EDCs) – MEDIATED OXIDATIVE STRESS IN WATER FLEA *DAPHNIA MAGNA* H Y Kim and <u>Y M Lee</u>

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Heavy metals have adverse effects to aquatic organisms, and endocrine disrupting chemicals (EDCs) alter normal hormonal functions in living organisms. In particular, heavy metals can produce reactive oxygen species (ROS), and lead to oxidative stress under chronic condition. Daphnia magna is a representative model species in ecotoxicology and toxicogenomics due to their sensitivity to many kinds of chemicals. However, molecular studies on these chemicals-induced oxidative stress are still lack in this species. In the present study, acute toxicity test was performed to determine EC_{50} values in D. magna exposed to Cd, Pb, Hg, 4-NP and BPA for 48h, according to OECD test guideline 202. Total GSH contents and GST activity were measured, and the mRNA expression level of oxidative stress markers (four glutathione S-transferase (GST) isoforms, glutaredoxin (Grx), glutathione peroxidase (GPx) and thioredoxin reductase (TrxR)) was analyzed by real time RT-PCR after chemical exposure. As results, all chemicals showed a negative effect on mobility. The 48h - EC₅₀ values were 21.0 µg/L for Cd, 694.6 µg/L for Pb, 3.8 µg/L for Hg, 18.9 µg/L for 4-NP and 8.3 mg/L for BPA. The order of toxicity based on EC₅₀ values was Hg> 4-NP> Cd > Pb > BPA. Total GSH contents and GST activity was significantly induced in chemical exposed group. A significant modulation of oxidative stress marker genes was observed after exposure to each chemical with different expression patterns depending on kinds of chemicals. Most of them, GST sigma gene expression level was the highest. These findings imply that these chemicals could induce oxidative stress in D. magna, and oxidative stress markers, in particular GST sigma seem to be actively involved in cellular protection against chemical-induced oxidative stress. This study would helpful to understand molecular mode of action of heavy metals and EDCs in water flea.

DETOXIFICATION – RELATED GENES IN MARINE CILIATE, *EUPLOTES CRASSUS*: MOLECULAR CLONING AND CHARACTERIZATION IN RESPONSE TO ENVIRONMENTAL POLLUTANTS

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Detoxification system including cytochrome P450 monooxygenase (CYP450) and ATP-binding cassette (ABC) transporter are responsible for defence against toxicity of environmental pollutants, such as heavy metals and polycyclic aromatic hydrocarbon (PAHs), as well as endogenous reactive metabolites. Marine ciliate Euplotes crassus, a single-cell eukaryote have been considered as a model organism for monitoring of environmental pollutions in sediments. However, study on cellular defence system at molecular level is still lack in ciliate. Here, we identified and characterized five CYP450 genes (Ec-CYP450), and four ABC transporter (Ec-ABC) genes in E. crassus. Gene expression was analysed by real time RT-PCR, and luciferase assay for CYP450 and efflux test for ABC transporter were further performed after exposure to cadmium (Cd), and/or benzo[a]pyrene (B[a]P). As results, Ec-CYP450 family genes had conserved domains and clustered with those of the order Hypotrichia in phylogenetic analysis. Five Ec-CYP450 genes were significantly up regulated in response to B[a]P, while Ec-CYP450 activity was not changed. Luciferase assay in mammal cell transformed vector with promoter of Ec-CYP450 showed a significance induction after B[a]P exposure. These findings suggest that Ec-CYP450 may be involved in detoxification of B[a]P. In case of Ec-ABC transporter, highly conserved transmembrane helices were found. Their gene expression level were significantly elevated with different expression pattern. Efflux test showed an inhibition of Ec-ABC transporter function in concentration-dependent manner after exposure to Cd and B[a]P. Our findings indicate that Ec-ABC transporter may be involved in detoxification of these chemicals, and play an important role in cellular defence against Cd and B[a]P - mediated toxicity. This study would be helpful to develop molecular biomarkers for monitoring environmental pollutions and to understand molecular mode of action in the ciliate exposed to these toxic substances.

DETERMINATION OF PHTHALATE ESTERS IN HARBOR SEDIMENTS BY CAPILLARY GAS CHROMATOGRAPHY AND MASS SPECTROMETRY DETECTOR

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Phthalate esters (PAEs) were organic endocrine disruptors and/or environmental hormones that may mimic or interfere with endocrine. Analysis of 10 PAEs was carried out in sediment samples of Kaohsiung Harbor of Taiwan which is supposed to be extensively polluted by municipal and industrial wastewater discharges. The determination and quantification of PAEs in sediment samples were performed using gas chromatography/ mass spectrometry (GC/MS) with the aid of two internal standards and two surrogate standards. Results of the average concentration of total PAEs (ΣPAE_{10}) in the harbor sediment was 7,714±21,763 ng/g dw (812 and 99,342 ng/g dw) with di-(2-ethylhexyl) phthalate (DEHP) and Diisononyl phthalate (DiNP) as the major species that constitutes 49.9% and 48.9% of ΣPAE_{10} found in the sediment. The spatial distribution of PAEs revealed that PAEs concentration was relatively higher near the river mouth regions, especially in the Love river mouth where it gradually diminishes toward the harbor region. This indicates that major sources of pollution originate from the upstream municipal and industrial wastewater discharges. As compared with sediment quality guidelines, the observed levels of DEHP in the Kaohsiung Harbor exceeded the threshold effect level (TEL), which will eventually cause acute biological damage. Moreover, the levels of DEHP in the river mouth areas exceeded the probable effect level (PEL). This indicates that the concentration of DEPH found in Kaohsiung harbor sediment may cause adverse impact on aquatic lives.
DOES ENERGETIC COST FOR LEAF CONSTRUCTION IN *SONNERATIA* CHANGE FROM ORIGINAL TO INTRODUCED SWAMPS AND DIFFER FROM OTHER MANGROVE GENERA IN SOUTH CHINA?

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Leaf construction cost (CC) and other growth traits of two Sonneratia species (Sonneratiacaseolaris and S. apetala) and four native mangroves (Bruguieragymnorrhiza, Kandeliaobovata, Aegicerascorniculatum andAvicennia marina) between their original region (Hainan) and the introduced place (Shenzhen) were compared to evaluate the invasive potentials of Sonneratia in the new habitat. No significant differences in leaf CC and growth traits of six mangroves between Hainan and Shenzhen mangrove swamps were found while these indices differed significantly among six species, according to two-way analysis of variance (ANOVA). CC per unit mass (CCM), CC per unit area (CCA) and caloric values of Sonneratia species were significantly lower than those of native mangroves while the specific leaf area (SLA) were just the opposite. The CCM values of S. caseolaris and S. apetalawere 6.07% and 11.87% lower than those of native mangroves, respectively. The CCM values sequences of six mangroves in the two swamps followed the same order, that is, S. apetala S. caseolaris A. marina B. gymnorrhiza K.obovata< Ae. corniculatum. These findings indicated that Sonneratiadid not exhibit more invasive potentials in the new habitat compared to their original one. The lower CC and higher SLA of Sonneratia in both swamps suggested this genus had higher invasive potentials than the natives. The lower CCM of S. apetalathan S.caseolaris further implied that the former species of Sonneratia was more invasive than the latter one, and the same CCM values orders among six mangroves indicated that CCM may be an inherent trait to evaluate the invasiveness of an alien species.

TRACE METALS AND STABLE LEAD ISOTOPES IN TISSUES OF FISHES COLLECTED FROM YONGSHU REEF, SOUTHERN SOUTH CHINA SEA <u>Y Xu^{1,2}, M G Cai^{1,2}, H W Ke², M Chen², Y Lin², H X Deng² and M J Ni²</u>

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Fish can be always used as an indicator for toxic substances presented in aquatic environment. Trace metals and Pb isotopes were measured from tissues of five fish species from the coral area in the southern South China Sea (SSCS) in the summer of 2013, to assess the level of trace metals, their potential ecological risk and to trake their sources. The concentrations of trace metals in the tissues (dry weight) were ND-21.60 mg/kg (Cd), 1.21-4.96 mg/kg (Cr), 0.42-76.5 mg/kg (Cu), 0.92-103.96 mg/kg (Mn), 0.30- 29.97 mg/kg (Ni), 6.04-1857.15 mg/kg (Zn), 14.89- 6689.70 mg/kg (Fe), 0.22-3.34 mg/kg (Pb), and 0.03- 0.42 mg/kg (Hg), respectively. The hierarchy of the concentrations in the muscle is as follows, Cd<Hg<Ni<Pb<Cu<Cr<Mn<Zn<Fe. The metal concentrations of the most samples are lower than the allowed value of GB 2762-2012 (China) and FAO (USA), which suggest that human consumption of these species may cause less health risk. The liver and intestine absorbed more metals than the muscle, gill and gonad. This is probably because that the liver and kidney are detoxification organs. For he gill, the concentrations of Mn and Pb were higher than other tissues, while relatively higher Cr, Zn and Cu were found in the gonad. About the stable lead isotope ratios, the data of the ²⁰⁸Pb/²⁰⁷Pb and ²⁰⁷Pb/²⁰⁶Pbin fishes were different from those found in south of China, indicating that influence of terrigenous input from southern China mainland might be insignificant for the SSCS.

PHYSIOLOGICAL STRESS RESPONSE AND GROWTH REGULATION IN RESPONSE TO SHORT-TERM SUBLETHAL AMMONIA EXPOSURE IN GROUPER (*EPINEPHELUS COIOIDES*)

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Ammonia is a common pollutant has high risk of toxicity to the physiological functions of aquatic animals. In this study, to explore the potential influence of short-term ammonia exposure on stress response and growth physiology in an economically important mariculture species, orange-spotted grouper (*Epinephelus coioides*) were exposed to two sublethal concentrations of ammonia (0.2 and 0.4 mM) for 3 days to examine organismal stress indicators, transcriptional and hormonal responses of growth hormone (GH)/insulin-like growth factor 1 (IGF-1) axis as well as genes involved in muscle growth regulation. The results showed the significant decrease and gradually increase in plasma glucose and cortisol levels were observed, respectively. In growth regulation responses, transcript levels of pituitary GH, hepatic IGF-1, GH receptor 2 (GHR2), muscle myosin heavy chain (MHC), myostatin, IGF receptors (i.e., IGFRa and IGFRb) were down-regulated in grouper exposed to sublethal ammonia. However, liver GHR1 mRNA expression, plasma GH and IGF-1 levels were not significantly affected. Taken together, short-term sublethal ammonia exposure would induce physiological stress response and disturb transcriptional regulation involved in GH/IGF-1 axis and muscle growth in grouper. This study will be helpful to understand the potential negative effect of waterborne ammonia on growth physiology in fish.

MOLECULAR AND CELLULAR STRESS RESPONSES TO SUBLETHAL AMMONIA EXPOSURE IN WHITE SHRIMP: PROTEIN QUALITY CONTROL, ANTIOXIDANT MECHANISM AND APOPTOSIS

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The intensive and recirculating aquaculture systems are common practices nowadays which easily lead to rising waterborne ammonia to toxic levels to aquatic animals. Pacific white shrimp (Litopenaeusvannamei) is an important worldwide aquaculture species. However, study on the mechanisms of molecular and cellular stress responses employed for coping with sublethal ammonia stress remains limited. In this study, regulatory responses of protein quality control (PQC), antioxidative mechanisms and apoptosis were investigated in shrimp gills after 96h exposure to sublethal ammonia levels (1.5 and 3 mg/L TAN). In PQC mechanism, elevation of mRNA levels of heat shock factor 1 (HSF1)at 3 mg/L TAN and heat shock protein 70 (HSP70) in two ammonia treated groups were foundwhile the amounts of aggregated proteins was similar among all the groups. Moreover, the transcript levels of antioxidative enzymes, manganese superoxide dismutase (Mn SOD), glutathione peroxidase (GPx) and catalase evidently upregulated at3 mg/L TAN exposure. However, no significant difference was found in total antioxidant capacity among all groups, possibly implying the antioxidative enzymes acted a predominant role in antioxidant cytoprotection in white shrimp under ammonia stress. In addition, the results from apoptotic responses revealed that transcript levels of caspase-3, -4 and -5 increased as well as extensive DNA fragmentation was induced obviously in shrimp gills exposed to 3 mg/L TAN. This study improved the understandingsof vital cytoprotective roles of PQC, antioxidativedefenece and apoptosis in coping with sublethal ammonia stress in white shrimp. These findings were suggested to be helpful in predicting sublethal ammonia toxicity and potentially useful in environmental monitoring studies.

DIFFERENCES IN LITTER ECOLOGICAL CHARACTERISTICS BETWEEN NATURAL AND PLANTED KANDELIA OBOVATA MANGROVE FOREST IN FUTIAN SHENZHEN, CHINA

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Litter ecological characteristics in natural (*Kandelia obovata*) and plantations (*Kandelia obovata*) mangrove forests in Futian, Shenzhen, China were compared from 2010 to 2015. The Natural forests (*Kandelia obovata*) had significantly higher litter production than the plantations (*K. obovata*), with mean annual total litter of 15.3 t ha⁻¹ yr⁻¹ and 14.2 t ha⁻¹ yr⁻¹, respectively. The plantations had significantly higher energy flow than that of natural forests, with mean annual energy flow of 16.3 KJ/m² and 15.7 KJ/m², respectively. The nutrient composition of organic carbon content in natural forest is higher than the plantation, total nitrogen and total phosphorus content in plantation is significantly higher than those of the natural forest. These results suggest that the ecological function of mangrove litter and better understood the significance of litter fall in mangrove restoration.

APPLICATION OF ELECTRO-COAGULATION-FLOTATION METHOD ON HARVESTING MICROALGAE IN ARTIFICIAL MEDIA AND WASTEWATER

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The release of domestic or industrial wastewater can cause contamination of our water bodies and cause algae bloom. In our study, Electro-coagulation-flotation (ECF) technology was used for harvesting microalgae strain *Chlorella vulgaris* in artificial culture media and wastewater to investigate its harvesting efficiency.

Electro-coagulation-flotation (ECF) is the application of electrochemical reaction in the electrode and the dispersion of hydrogen gas to neutralize and allow floatation of microalgae cell for surface harvesting.

A lab scale ECF harvester was designed on harvesting microalgae. The harvester composed of a harvesting tank, DC power supply, overhead stirrer and flat material plates.

Different ECF parameters such as pH, salinity, operation time, operation current and voltage was investigated, adjusted and optimized for increasing the harvesting.

Furthermore, the harvesting alga was re-cultivated in artificial medium BG-11 for 14 days to study its growth rate and the changes of its chlorophyll and lipid content.

ECF efficiency was shown to be more efficient in aluminium electrode than iron and carbon electrode. The ECF process could be optimized by reducing the initial pH of the culture being harvested. Although a higher current density with higher voltage supply resulted in a rapid ECF process with a higher flocculation speed, but it would increase the cost of the harvesting method and disturb the cell nature of the microalgae. The microalgae harvested from high density could not be cultivated again rapidly in artificial culture media with losing chlorophyll pigment and low cell division rate. Total lipid productivity was determined in the ECF process and it is closely related to the harvesting efficiency. Under optimal condition, the harvesting efficiency of the process can be increase significantly and reduced the operation cost.

ECF harvesting method is particular efficient in high salinity water because of the higher conductivity in the culture and lower power consumption. It is a promising approach on harvesting microalgae in waste or marine water to become one of the control method for algae bloom.

PERFORMANCE AND BACTERIAL COMMUNITY STRUCTURE OF MANGROVE CONSTRUCTED WETLAND UNDER TEN-YEAR OPERATION

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This study aimed to evaluate the purification effect of a horizontal subsurface-flow mangrove constructed wetland which has been operated for 10 years, and to isolate the dominant groups of bacteria which are associated with the removal of pollutants from the sewage input to the system. The constructed wetland was consisted of three independent belts, one without vegetation as the control whereas the other two being planted with mangroves Aegiceras corniculatum and Kandelia obovata separately. One-year monitoring showed that biochemical oxygen demand (BOD) and total phosphorus (TP) removal efficiencies were similar in the two planted belts, with 32.3% - 80.0% for BOD and 32.3% - 95.2% for TP, respectively. However, the chemical oxygen demand (COD) and total nitrogen (TN) removal efficiencies in the A. corniculatum belt were significantly higher than that of K. obovata (p<0.05). A high abundance of ammonifiers, nitrifiers and denitrifiers was recorded in rhizosphere soil samples from the planted belts, while high numbers of total bacteria were obtained from the unplanted belt, especially in summer, with average 3.9×10^6 CFU g⁻¹ as compared to 2.4×10^6 CFU g⁻¹ in the A. corniculatum and 2.9×10^6 CFU g⁻¹ in the K. obovata belts. Bacterial community structure was investigated using denaturing gradient gel electrophoresis (DGGE). The Shannon-Weaver diversity index, calculated from the DGGE profiles showed that the bacterial diversity were significantly higher in the unplanted belt, but not in the planted belts (p<0.05). Cluster analysis of bacterial profiles further revealed that the bacterial isolates retrieved from the three belts were related phylogenetically to Acidobacteria, Proteobacteria, Firmicutes, Actinobacteria, Gemmatimonadetes, Chloroflexi and Cyanobacteria. At least seven common dominant groups of microorganisms were found in the rhizosphere of both mangrove plants, and five of them had close relationship with denitrifying bacteria. Thus, the bacterial community structure in this ten-year old mangrove constructed wetland appeared to be simple, and the predominating bacterial groups were mainly correlated with the denitrification process in sewage treatment.

TOXICITY AND REMOVAL OF PHENANTHRENE, FLUORANTHENE AND PYRENE BY TWO *NAVICULA* SPECIES, A COMMERCIAL SPECIES (*N. INCERTA*) AND A LOCAL ISOLATE

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The tolerance and biodegradation ability of two Navicula diatoms, a local isolate (Navicula sp.) and a commercial species (Navicula incerta), to three polycyclic aromatic hydrocarbons (PAHs), phenanthrene (PHE), fluoranthene (FLU) and pyrene (PYR) at three incubation periods (1, 4 and 7 days) were studied. Both species were capable to remove PAHs, but the efficiencies were somewhat PAH-dependent and species-specific. The biodegradation percentage of FLU in N. incerta was the highest (34.15%), followed by PHE (29.94%) and PYE (24.31%) had the least degradation; while the cellular uptake of FLU (20.78%) was significantly lower than that of PYE (36.59%) and PHE (31.09%). On the other hand, the biodegradation percentage of PHE in the culture of Navicula sp. was the highest (50.39%), significantly more than FLU (29.88%) and PYE (18.82%). Similar to N. incerta, the uptake of PYE (48.66%) by cells of this local isolate was the highest. At the end of 7-days incubation, biodegradation percentages of PAHs in cultures of both Navicula sp. and N. incerta were the highest, significantly more biodegradation than those of the end of 1-day and 4-days incubation; while temporal changes of the residual amounts of PAHs remaining in the medium were the opposite. Median effect concentration (EC₅₀) values of the local isolate (Navicula sp.) to PHE and FLU were significantly lower than those of the commercial species (N. incerta), suggesting the local isolate was more sensitive to the toxicity of PHE and FLU than the commercial species. These results indicated that the local isolate did not exhibit a higher tolerance to PAHs and did not achieve better removal and biodegradation than the commercial species, which were different from the assumption that local isolate obtained from polluted environment would be more adaptive and suitable for bioremediation purposes than the commercial species

HEAVY METAL POLLUTION AND ASSESSMENT OF RESTORATION EFFICIENCY IN A RESTORED MANGROVE WETLAND: A CASE STUDY AT SHENZHEN WATERLANDS RESORT

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With the rapid development of society and economy, human activities in coastal area exerted high pressures on coastal ecosystems. Coastal wetland, as one of the most important coastal ecosystems, has been impacting heavily by human activities for decades. It causes the degradation of the coastal wetland, which has become an important issue in the world. The restoration of wetlands thus attracts attentions of different countries, and numerous projects have been carried out to restore the degraded wetlands. The restored coastal wetland, especially the urban wetland, usually located in the special economic zones and the regions of sensitive to the climate change. Consequently, structure and function of the coastal wetlands are always in a dynamic equilibrium process and, are fragile to the external pressures. Thus, it is quite necessary to evaluate the efficiency of the restoration at coastal wetlands, which is benefit for their management and functional maintenance. In this study, a restored mangrove wetland, i.e. mangrove forest-aquaculture coupling ecological restoration system, in Water Resort Park at Shenzhen was selected. Environmental monitoring was conducted in Mar. 2013, Mar. 2014 and Aug. 2014. All the environmental samples were collected in different sampling sites including mulberry base pond, exhibition Park and the mangrove forest base, which were constructed in 2008, 2004 and 2002, respectively. The severely polluted river outside of the restoration area was selected as a negative reference, while a mulberry base pond without mangrove was selected as contrast. The investigation of environmental quality included the water, sediment under mangrove. Comparison among results and the national water & sediment quality standards, and also the previous data in the same area were performed.

The results showed that the pH values, dissolved oxygen (DO), nutrients and heavy metal concentrations in the water of restored sites are in a better state than those in river water. The concentrations of all heavy metals in the restored ponds were all significantly lower than those of other aquaculture ponds adjacent to Pearl River Delta. These results demonstrated that the restoration of mangrove wetlands could significantly improve the quality of water environment. The concentrations of heavy metals in the sediment are also significant lower in restoration sites than those in the river site. It indicated that the growth of mangrove might effectively remove the heavy metals in the sediment. Based on the above results, the restoration of mangrove wetlands in Water Resort Park did achieve the initial objectives, including the protection of the ecological environment and the water purification for ecological aquaculture. The results of this study could be helpful in the evaluation of effect of ecological restoration of coastal wetland, and provide some recommendations on the management of coastal wetlands.

RECOVERY OF LITHIUM FROM SEAWATER WITH TITANIUM OXIDE ION-SIEVES

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The potential lithium resource in seawater can be as high as 2.5×10^{14} kg. A feasibility study for the recovery of lithium from seawater with protonated lithium titanium oxide ion-sieves prepared by the solid-state reaction method was, therefore, carried out in this work. Lithium ions (Li⁺) having a similar ion density with the H⁺ sites on the ion-sieves were exchanged with a high selectivity, and vice versa for their regeneration. By Li⁷-nmr, XRD, and EXAFS, it is clear that the exchange between H⁺ and Li⁺ has little perturbation on the chemical structure of the titanium oxide ionsieves. Note that the ion-sieve has negligible titanium dissolution during exchanges. After three cycles, the ion-sieve can sustain 85% of its best performance. The titanium oxide ion-sieves also have other applications in the areas of selective capture of metal ions from wastewater.

TWO CASES STUDY OF OIL SPILL CUE BLOOMING ALONG COASTAL OF KENTING NATIONAL PARK, SOUTHERN TAIWAN

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In 2009, the container ship Colombo Queen grounded off Jialeshui, and the oil tanker W-O BUDMO grounded off Houwan, both in southern Taiwan. Water quality was monitored to evaluate the environmental impact caused by these two events. The results show that the PAHs, ammonia, turbidity, and nutrient concentrations increased at the beginning but decreased to the background levels. On the other hand, DO saturation, pH and chl. a decreased at beginning, increased to maxima after 10 days, but recovered to the background levels by 14 days. The chl *a* concentration, pH and DO saturation fluctuated in a similar pattern during the oil spilled, implying that algal blooms were occurring. The fluctuation patterns of water quality were similar between the two events. In this study, we documented a full environmental recovery at coastal area before, during and after an oil spill.

TRIBUTYLTIN TOXICITY IN REEF-BUILDING CORAL (ACROPORA SPP.) ASSESSED BY MOLECULAR RESPONSES OF ANTIOXIDANT ENZYMES AND OOGENESIS RELATED GENES

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The harmful effects of organotins have raised a great concern. Among organotins, tributyltin (TBT) is a common marine pollutant known as an endocrine disruptor possesses high toxicity towards marine organisms. However, the information on TBT toxicity in coral was limited so far. To evaluate the potential physiological disturbances of reef-building coral (*Acropora* spp.) posed by TBT, the levels of accumulated organotins as well as molecular responses of antioxidant enzymes and oogenesis related genes were explored. After 7-day exposure to TBT at 0.5 and 1.0 ppb, the levels of accumulated organotins in coral increased with waterborne TBT concentrations. Furthermore, compared to control group, the transcript amounts of Cu/Zn and Mn superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx) elevated in coral under high TBT treatment. To pay attention to reproductive toxicity of TBT, genes of aromatase-like isoforms, estrogen receptor (ER) and two yolk proteins (vitellogenin; VTG and egg protein; EP) were examined. Expression of these oogenesis related genes significantly downregulated except ER in coral exposed to high TBT concentration. To our knowledge, this is the first study to assess TBT toxicity in coral at molecular level to provide more scientific data for realizing the potential threats of TBT to coral.

CORRELATING DEHALOCOCCOIDES ACTIVITY WITH IN-SITU ANAEROBIC BIOREMEDIATION EFFICIENCY ON TCE-CONTAMINATED GROUNDWATER

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Chlorinated solvent, when existing underground, the source zones and plumes are common threatening to groundwater quality. Trichloroethylene (TCE) is one of the most ubiquitous halogenated organic compounds. The related contaminants are of great concern because of their carcinogens, which lead to the stringent maximum allowable concentration levels in groundwater. Cost-effective approaches for remediation of aquifers contaminated by chlorinated volatile organic compounds (Cl-VOCs) include a link between Dehalococcoides (Dhc) bacteria and chlorinated ethene detoxification. To provide useful diagnostic tools for bioremediation monitoring, a quantitative real-time PCR(qPCR) approach targeting 16S rRNA genes and three Dehalococcoides reductive dehalogenase (RDase) genes(*tceA*, *bvcA*, and *vcrA*) was applied.

An eight-month groundwater bioremediation was conducted with injection of emulsified oil substrate (EOS) for a TCE-contaminated site. Laboratory experiments were conducted to assess microbial reductive dechlorination between one injection well with 25-meter depth (2A) and one monitoring wellwith 50-meter depth(5A).5A well was one-meter located directly down-gradient from the 2A well. About fifty days later, TCE in both of the two wells were below the regulation standard (0.050 mg/L). One hundred days later, an anaerobic environment was observed and this environment contributed to dominated *Dehalococcoides sp.*($10^8 \sim 10^9$ copies/L). The TCE concentration in 2A found to be negatively correlated with the Dhc concentration (r = -0.69). The critical performance once after 100 days has been emphasized in relevant studies. After 145 remediation days, RDase gene *tce*Aattained to 10^7 and 10^{10} cells/L in 5A and 2A well, respectively. Particularly *tce*A in 2A well reached 130 folds than it used to be.Before the EOS injection was conducted, *Dehalococcoides sp.* was only $10^5 \sim 10^6$ copies/L and none of the RDase genes were detected.

Paralleled to the degradation of TCE, cis-1,2-dichloroethene (cis-DCE) and vinyl chloride(VC) were observed as the by-products. During the entire remediation course, in2A well, the correlation coefficient between TCE and cis-DCE achieved 0.82. Finally the TCE degradation in 2A was 77%, which reduced the initial concentration of 0.0608 to the final 0.0142 mg/L. The degradation in 5A found even higher as 93%, which decreased the initial one of 0.151 to the final 0.0104 mg/L. The cause of the relatively high degradation rate in 5A can be explained as two possibilities: (1)the substrate in 5A was only one-tenth of that in 2A, so the substrate was better utilized in 5A than in 2A, and (2) the environment in 5Awas adequately anaerobic so that Dhc and dissolved oxygen was negatively correlated (-0.78). The correlation coefficient between Dhc and dissolved oxygen was relatively less negative (-0.45). In conclusion, use of qPCR to monitor dynamic of Dhcin an in-situ TCE contamination site provides important information of the microbial activity and the associated contributions during the bioremediation course.

ChemTHEATRE— A PLATFORM TO MANAGE THE MONITORING DATA OF CHEMICALS IN ENVIRONMENT

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We are developing a platform to deposit and visualize the monitoring data of chemicals in environment, named ChemTHEATRE (Chemical in the THEATRE: <u>T</u>ractable and <u>H</u>euristic <u>E</u>-<u>A</u>rchive for <u>T</u>raceability and <u>R</u>esponsible-care <u>E</u>ngagement). ChemTHEATRE stores chemical concentrations in environmental specimens with metadata of samples (sampling date and location, organism and its biometry, and so on) and of experimental methods (extraction method, used standards, instruments, etc). This platform enables us to visualize temporal and spatial distribution pattern of registered contaminants, and also to simulate their behavior and fate in the environment. Additionally, using this platform, you may directly reach the specific environmental specimens stored in the environmental specimen bank (es-BANK) at Ehime University, which allows us to obtain some additional data from the same samples, if necessary. Please visit ChemTHEATRE (http://chem-theatre.jp/), free to access. We will also appreciate if you would kindly contribute to ChemTHEATRE to register your published monitoring data.

METAGENOMIC PROFILE OF POLYCYCLIC AROMATIC HYDROCARBONS DEGRADATION RELATED GENES BETWEEN PEARL RIVER ESTUARY AND SOUTH CHINA SEA

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Pearl River Estuary, with connects the Pearl River with the South China Sea, receives amount of pollutions from the Pearl River Delta by Pearl River input. It becomes an important depositional site for organic contaminations including polycyclic aromatic hydrocarbons (PAHs). High concentration of PAHs in sediment of Pearl River Estuary would influence the microbes in situ. This study aimed to investigate the effect of PAHs on bacterial community structure and PAHs degradation related genes by metagenomics technology.

Two sediment samples were collected from Pearl River Estuary and six samples were collected from South China Sea in contrast. For every sample, PAHs concentration, bacterial 16S rDNA and PAHs degradation related genes abundance were analysed. The results showed that the PAHs concentration and PAHs degradation related genes abundance were both higher in Pearl River Estuary than that in South China Sea. By 16S rDNA analysis, published PAHs degradation bacteria were recognised. The proportion of PAHs degradation bacteria was also higher in Pearl River Estuary (14.15-15.39‰) than South China Sea (4.60-12.32‰). This suggested that high concentration of PAHs could promote the enrichment of PAHs degradation bacteria and PAHs degradation related genes.

DEGRADATION PATHWAYS OF 1-METHYLPHENANTHRENE IN BACTERIAL SPHINGOBIUM SP. MP9-4 ISOLATED FROM PETROLEUM-CONTAMINATED SOIL

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Alkylated polycyclic aromatic hydrocarbons (PAHs) are abundant in crude oil, and alkylated phenanthrenes are considered as the primary PAHs during some oil spill events. However, little attention has been focused on their removal and biodegradation in the environments. Bacterial strain of *Sphingobium* sp. MP9-4, isolated from petroleum-contaminated soil, was efficient to degrade 1-methylphenanthrene (1-MP), from 9.24 to 0.39 mg/L over 2 days. A detailed metabolism map of 1-MP in this strain was delineated based on analysis of metabolites using GC/MS and degradation pathways of PHE by strain MP9-4 as the reference. 1-MP was initially oxidized via two different biochemical strategies, including benzene ring and methyl-group attacks. Benzene ring attack was first involved with dioxygenation and *meta*-cleavage of the non-methylated aromatic ring via similar degradation pathways of PHE by bacteria. The oxidation of methyl-group led to the formation of alcohol and carboxylic acid. Production of 6-methylsalicyclic acid and salicyclic acid suggests that *Sphingobium* sp. MP9-4 can assimilate 1-MP by aromatic ring hydroxylation and by the oxidation of methyl side chain. This study enhances the understanding of the metabolic pathways of alkylated PAHs and shows the significant potential of *Sphingobium* sp. MP9-4 for the bioremediation of alkylated PAHs contaminated areas.

MODULATING EFFECT OF EXUDATES RELEASED BY KELPS ON THE COPPER TOXICITY IN MARINE ORGANISMS. POTENTIAL USE IN THE ECOLOGICAL RESTORATION OF COASTAL AREAS POLLUTED BY COPPER MINE TAILINGS

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The brown kelp Lessonia sp. (Laminariales, Phaeophyceae) is a key dominant species along of the Southeast Pacific coast. It forms extensive kelp beds of significant ecological importance, providing habitat for many seaweeds, invertebrates and fishes in the intertidal zones of wave-exposed rocky shores along the Pacific coast of South America. However, the role of the kelp could be affected by abiotic stress such as metals. It has been demonstrated that in order to counteract the toxic effect of metals, Lessonia sp. has the capacity to produce and release to the surrounding water, copper complexing ligands (exudates), even under stress by copper. On the other hand, there is a zone in the coastal of Atacama (Northern Chile) that has suffered disturbances due to copper mine activities. This is the case of Chañaral, where more than 150 million tons of copper mine tailings has been chronically discharged in the coastal zone, causing the disappearance of the brown kelp Lessonia berteroana (northern lineage of Lessonia nigrescens) considered as an ecosystem engineer, diminishing in consequence, the biodiversity on more than 20 km of coastline. In this context, the protective effect of exudates released by Lessonia sp. on spores of the same species and in early developmental stages of organisms ecologically related, such as spores of the green alga Ulva lactuca (Chlorophyta) and the larvae (zoea I) of the kelp crab Taliepus dentatus (Milne-Edwards), were determined by toxicological bioassays with copper, and with and without the presence of exudates in the culture media. The ecotoxicological assays showed that exudates increase the EC_{50} of spores' germination from 8.05 to 23.3 μgCu L⁻¹ in U. lactuca; and from 119 to 213 μgCu.L⁻¹ in Lessonia sp., Also, exudates increase the LC₅₀ of zoea I crab larvae from 144.2 to 249.2 µgCu.L⁻¹. These data reveal that the spores of both considered species, and in the presence of kelps exudates, could germinate, and continue with its development; in the mentioned environment with high levels of dissolved copper. The coastal area of Chañaral, display variable but persistently high concentrations of dissolved copper in the seawater (from $8.7\mu g L^{-1}$ to $34.1\mu g L^{-1}$), with a severe reduction in species richness and a complete modification of the intertidal community structure. The present study demonstrates that the brown kelp Lessonia's exudates have a protective role on different species of algae and invertebrates associated with the kelp ecosystem, specifically to early stages of the life cycle, such as spores, microscopic gametophyte and sporophytes of algae and larval stages of invertebrates. Furthermore, Taliepus dentatus's larvae (Zoae I) seem to be potentially adapted and able to live in the area with high copper levels. More questions arise about the possible restoration of the copper enriched ecosystem in the long-term, if copper concentrations in the water decrease and algal exudates increase, which will allow the quantification and qualification of changes in the biodiversity of the coastal ecosystem.

TARGET TISSUES OF THE MANILA CLAM *RUDITAPES PHILIPPINARUM* FOR STUDYING METAL ACCUMULATION AND BIOMARKERS IN ENVIRONMENTAL MONITORING

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To characterize the target tissues of the manila clam Ruditapes philippinarum for use as test organisms for environmental study, the accumulation of trace metals and three biomarkers were measured in different organs. Exposure with Cu and Pb carried out under laboratory conditions revealed a linear uptake of metals throughout the experimental period in each tissue. In particular, significant increase was observed in gills and mantle. The increase of intracellular reactive oxygen species showed the great potential of gills as a target tissue for both Cu and Pb exposure. The highest activity of glutathione-S-transferase and their relative increase in activity were also observed in gills. Metallothionein-like protein levels, however, increased greatly in the digestive gland and mantle during Cu and Pb exposure, respectively, although all tissues, except the foot, showed significant changes after 24 h of metal exposure. In the field study, the highest concentration of metals was recorded in the gills and mantle, accounting for over 50% of the total accumulated metal in all sites. Additionally, Cu and Pb increased significantly in these two organs, respectively. However, the order of accumulation rate in laboratory exposure was not concomitant with those of the lab-based study, suggesting that different routes of metal uptake and exposure duration induce distinct partitioning of metals and regulating system in R. philippinarum. These series of exposure studies demonstrated that gills, mantle, and digestive gland in R. philippinarum are potential target tissues in environmental monitoring study using metal concentrations and biomarkers.

COMPARISON OF QUANTITATIVE PCR AND TRADITIONAL CULTURE-BASED METHODS FOR ENUMERATION OF *ESCHERICHIA COLI* IN SHING MUN RIVER

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Rapid enumeration of faecal indicators is crucial for water pollution control under emergency conditions. The quantitative polymerase chain reaction (qPCR) could be a competent alternative to culture-based method by its fast turnaround time, but the validation studies using local cases are few. This investigation evaluated the use of qPCR for monitoring of *E. coli* in Shing Mun River. Densities of *E. coli* in river water samples collected from September 2014 to March 2015 were determined using both qPCR and culture-based methods. Regression analysis showed that the two methods had a significant positive linear relationship with a correlation coefficient (r) of 0.68. The amounts of *E. coli* measured using qPCR were significantly higher than those using culture-based methods. This study showed that the qPCR has a potential to complement the traditional culture methods for rapid assessment of faecal pollution in local river water.

CAPACITIVE DEIONIZATION OF SEAWATER WITH DISINFECTION ABILITY BY Ag@C/rGO ELECTRODES

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The scarcity of clean drinking water has been a serious issue with the increasing world population and climate change. Capacitive deionization (CDI) being the key method in seawater desalination has the advantages of low energy consumption, economical and environment friendly. In the present work, the Ag@C core-shell nanoparticles on graphene nanosheets (Ag@C/rGO) was prepared and used for CDI of seawater with the antibacterial ability. The Ag@C/rGO electrodes were characterized by SEM, TEM, FTIR, Raman, BET surface area analysis, and cyclic voltammetry. Their chemical structure was also studied by component-fitted synchrotron X-ray adsorption near edge structure and small angle X-ray scattering (SAXS) spectroscopy. Experimentally, in a one through pass, the average removal efficiency of NaCl from seawater in the CDI process was 15%. Additionally, the disinfection efficiency was as high as 97% in a 4-h contact time. This study demonstrates that the Ag@C/rGO electrodes are very effective for CDI of seawater with additional disinfection ability.

DERIVATION OF MARINE WATER QUALITY CRITERIA FOR METALS BASED ON A NOVEL QICAR-SSD MODEL

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Establishment of water quality criteria (WQC) is one procedure for protecting marine organisms and their ecosystems. This study, which integrated two separate approaches, Quantitative ion characteractivity relationships (QICAR) and species sensitivity distributions (SSDs), developed and validated a novel QICAR-SSD model. The QICARs predict relative potencies of individual elements while SSDs integrate relative sensitivities among organisms. The QICAR-SSD approach was applied to derive WQC for 34 metals or metalloids in saltwater. Relationships between physicochemical properties of metal ions and their corresponding potencies for acute toxicity to eight selected marine species were determined. The softness index (σ p) exhibited the strongest correlation with the acute toxicity of metals ($r^2 > 0.662$, F > 5.877, p < 0.0938). Predictive criteria maximum concentrations for the eight metals, derived by applying the SSD approach to values predicted by use of QICARs, were within the same order of magnitude as the values recommended by the U.S. EPA (2009). In general, these results demonstrate that the QICAR-SSD approach is a rapid method for deriving scientifically defensible WQC for metals, for which little or no information is available for marine organisms.

NMR-BASED METABOLOMICS FOR THE ENVIRONMENTAL ASSESSMENT OF KAOHSIUNG HARBOR SEDIMENTS EXEMPLIFIED BY A MARINE AMPHIPOD (HYALELLA AZTECA)

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Aquatic sediment is a long-term sink of all pollutants, therefore it has played an important role in the biohydrologeochemical cycle of chemical species in the ecological environment. Inflow of waste water from upstream rivers bring large flux of pollutants entering the Kaohsiung Harbor daily. In order to reveal the ecological risk of Kaohsiung Harbor sediments, we have applied an ecological metabolomic approach to investigate environmental factors pertinent to physiological and metabolic pathways of a marine amphipod *Hyalella azteca*, at the molecular levels, after exposure to sediments collected from different location in the Kaohsiung Harbor including the stream inlet of four rivers of Love River (LR), Canon River (CR), Jen-Gen River (JGR), and China Steel Harbor (CSH). These rivers are flowing through region of high human and industry activities. Additional, an outside harbor (OH) was selected as a reference site. Adult female Hyalella azteca was exposed to different sediments in seawater. After 10-day exposure, the metabolomic analysis of Hyalella azteca was carried out using ¹H NMR spectroscopy and multivariate statistical analyses. Results of principal component analysis (PCA) and partial least square discriminant analysis (PLS-DA) revealed significant separation between extracts of Hyalella azteca as 2 groups: (OH, LR, CR), and(JGR, CSH). Results of pathway analysis showed that metabolites were significantly involved in (1) pantothenate and Co A biosynthesis, and pyrimidine metabolism in (OH, LR, CR); (2) glutathione metabolism, phenylalanine, tyrosine and tryptophan biosynthesis, tyrosine metabolism, and arginine and proline metabolism in (JGR, CSH). Results demonstrated that NMR-based metabolomics can be an efficient method for characterizing metabolic responses related to heavy metal contamination derived from polluted area.

PREVALENCE STUDY OF ANTIBIOTIC RESISTANCES IN ESCHERICHIA COLI ISOLATED FROM THE ENVIRONMENTAL WATER OF HONG KONG

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Often found in sewage discharged from agricultural and aquacultural sites, residential areas and pharmaceutical factories, antibiotics are emerging environmental pollutants. The selective stress imposed by antibiotics onto environmental microbiota would promote the development of antibiotic resistance. When such pathogens spread to the community, they have the potential to cause complications in diseases and threaten human health due to an increased difficulty in treating antibiotic resistant microbes. In regard of this, prevalence of antibiotic resistances in pathogenic indicator, *Escherichia coli (E.coli)*, was proactively screened in the Hong Kong environment.

A total of 94 *E. coli* isolates, sampled from Kam Tin River and Kai Tak Nullah during 2014-2015, were investigated for their susceptibility to 6 antibiotics, namely cefotaxime, ceftazidime, nalidixic acid, ciprofloxacin, tetracycline and ampicillin. Four antibiotic resistance genes, including those related to the production of extended spectrum of β -lactamase (ESBL) (*bla*_{CTX-M} and *bla*_{TEM}) and tetracycline-resistance (*Tet*A and *Tet*B), were also screened. A total of 30 *E. coli* isolates sampled from Silvermine Waterfalls were used as control to set up a baseline for bacterial antibiotics resistance.

Phenotypically, all 30 Silvermine Waterfalls isolates were found to be susceptible to all antibiotics tested. In addition, they do not bear any examined antibiotic resistance genes. Of 94 isolates sampled from Kam Tin River and Kai Tak Nullah, high prevalence of antibiotic resistance was found. Seventy-seven isolates (82%) showed phenotypic resistance to at least one examined antibiotics while 56 (60%) carried at least one of the antibiotic resistance genes tested. Ampicillin resistance is observed in around 66% of isolates, followed by tetracycline (65%), nalidixic acid (40%) and ciprofloxacin (17%). Around 9% of isolates were ESBL producers. Moreover, *bla*_{CTX-M}, *bla*_{TEM}, *Tet*A and *Tet*B were found in 8%, 41%, 49%, and 17% of the isolates respectively. Around 66% isolates showed resistance to more than one antibiotic and 3 isolates displayed resistance to all 6 tested antibiotics.

High prevalence of antibiotic resistance recorded in this study reflected that environmental water represents a huge reservoir of microbiota harboring antibiotic resistance genes. Should horizontal gene transfer occur among resistant bacterial populations, development of multi-drug resistance pathogens would be inevitable. It is therefore suggested that regular monitoring of antibiotic pollutions and antibiotic resistance prevalence should be implemented. Otherwise, a new level of challenges is likely to be anticipated by the public as diseases with stronger tolerability to therapies emerge.

The POTENTIAL IMPACT OF OCEAN ACIDIFICATION UPON EGGS AND LARVAE OF MARINE FISHES

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Anthropogenic carbon dioxide (CO₂) emissions are resulting in increasing absorption of CO₂ by the earth's oceans, which has led to a decline in ocean pH, a process known as ocean acidification. The potential effects of elevated CO₂ level in marine environment on marine fishes are still poorly known. In present study, we investigated the effects of ocean acidification on the embryonic development and hatching rate of eggs and larvae of the palette surgeonfish (Paracanthurus *hepatus*), the bluestriped angelfish (*Chaetodontoplus septentrionalis*) and the goldlined seabream (Rhabdosargus sarba) to different artificially acidified seawater (pH 7.7 and 7.4, respectively) was demonstrated, and compared with those in control group (pH 8.2). The results showed that CO₂driven seawater acidification (pH 7.7 and 7.4) had no detectable effect on hatching rate and hatching size (total length) of embryos. However, the yolk utilization rate of larvae in control treatment (pH 8.2) was significantly (p < 0.05) higher than that in CO₂-driven seawater acidification (pH 7.7 and 7.4). These results suggest that although *R. sarba* might be more tolerant of elevated CO₂ than *P. hepatus* and *C. septentrionalis*, the effect of elevated CO₂ level on the calcification of skeletal elements are likely to be the most susceptibly physiological process of pH regulation in early life stage of seabream. Despite the technical challenges with these experiments, there is a need for future empirical work which can in turn support modeling-based approaches to assess how ocean acidification will affect the ecologically and economically important marine fish resources.

EFFECTS OF pH ON *NITZSCHIACLOSTERIUM* AND *SKELETONEMACOSTATUM* GROWTH AND NH4-N UPTAKE

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Ocean acidification (OA) though the dissolution of anthropogenic CO₂ into seawater has raised extensive concern for marine environmental problems. And eutrophication is becoming increasingly serious with high concentration of ammonia nitrogen (NH4-N) in coastal-sea under anthropogenic impact. OA coupled with the NH4-N source effect in coastal waters is likely to affect the survival of phytoplankton. In this work, Nitzschiaclosterium and Skeletonemacostatum were chosen as typical species of diatom in Chinese coastal ecosystems to test the potential effect of ocean acidification. Furthermore, these two diatoms were incubated in NH₄-N source in laboratory at different pH values (8.24, 7.81, and 7.61). Results showed that the terminative biomass, the maximum growth rate, and the maximum uptake rate of Nitzschiaclosterium significantly decreased with pH decline; nevertheless, the three parameters of Skeletonemacostatum increased firstly and then decreased with pH decline, and the peak of the three parameters is at pH 7.81. The maximum uptake rate declines more than the maximum growth rate, which implies that more nitrogen is assimilated for *Nitzschiaclosterium* with pH decline, whereas the change trend for Skeletonemacostatum is opposite. Therefore, the inhibition for Nitzschiaclosterium growth at pH 7.61, which could be regarded as the coupling effect of OA and eutrophication, is more serious than Skeletonemacostatum growth; but it is beneficial for Skeletonemacostatum growth, against the inhibition for Nitzschiaclosterium growth to the contrary at pH 7.81 in the coast by the end of the 21st century.

RESPONSES OF CORAL CALCIFICATION TO ENVIRONMENTAL CHANGES: THE ROLES OF HOSTS AND SYMBIONTS

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Understanding how scleractinian corals respond to environmental changes at both global and local scales is an urgent issue in order to predict the future of coral reef ecosystems. Previous researches have demonstrated that corals are sensitive to various kinds of environmental factors, and the decreases of coral calcification have been reported in the current changing environments. However, the systematic information focusing on how corals could acclimatize to several levels of environmental changes is still limited. In this presentation, we introduce our previous and recent results of coral responses to environmental changes by mainly focusing on the aspect of coral calcification, which is one of the most important characters in coral life history and has been intensively studied by various approaches from biological and geochemical fields. In addition, we outline current information on acclimatization mechanism of corals to environmental changes (temperature, acidified seawater, nutrient, salinity, etc) from both aspects of coral hosts and symbionts. We also discuss future directions to further understand the responses of coral-algal holobiont to environmental changes and calcification mechanisms by using coral primary polyp system.

WHEN RELATIONSHIPS TURN SOUR: EFFECTS OF OCEAN ACIDIFICATION ON ECOLOGICAL INTERACTIONS

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Ocean acidification (OA) and warming caused by anthropogenic carbon dioxide has been widely indicated as a direct threat to marine biodiversity. Considerable research has now been completed on the genetic, physiological, and behavioral effects of OA on various species, particularly those that have calcifying structures. However, little has been done with regards to ecological interactions, a crucial topic for understanding population and community dynamics in acidified oceans. Typically, ecological interactions such as predation, competition, and parasitism, can have a significant compounding or alleviating effect under environmental stress. Our meta-analysis of the publications in the last fifteen years shows that around 2% of the literature on OA explicitly studied its effect on inter- or intra-specific interactions; among these significant interaction, habitat, taxonomic group and life stage bias exists in the species that have been studied. Using a simple box model (Nutrient, Phytoplankton, Zooplankton, Detritus NPZD model), we also demonstrated that predicted phytoplankton bloom dynamics differ between scenarios in which OA impacts were modelled for only producers, only grazers, or for both. Increase in phytoplankton growth due to increase carbon availability had no significant effect on chlorophyll concentration, in contrast, an OA-induced increase in zooplankton mortality prolong the phytoplankton bloom. Our work highlights the need to include a broader range of ecological interactions and model organisms in future ocean acidification studies in order to better predict potential shifts in ecosystem dynamics.

COMBINED EFFECTS OF OCEAN ACIDIFICATION AND COPPER ON SEA URCHIN LARVAL DEVELOPMENT

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We live in a multi-stressor world where oceans are impacted by both global (e.g. CO₂-driven climate change and ocean acidification) and local pressures. Coastal marine organisms are already challenged by anthropogenic stressors such as pollutants which may be magnified by the impacts of future global changes. For instance, ocean acidification is likely to change metal speciation as well as bioaccumulation and, consequently, metal toxicity. There are few information available on the effects of these interactions on marine biota. In this study, we provide insight on the combined effects of ocean acidification and copper pollution on the larval development of the sea urchin *Heliocidaris crassispina*. In laboratory culture, sea urchin larvae appeared quite robust to predicted pH changes as well as to high levels of copper contamination, albeit some morphological changes. The association of both stressors significantly increased abnormalities and respiration. Such interactions highlights the importance of understanding how organisms respond to co-occurring factors, including metal contamination and ocean acidification, in informing sound conservation management and remediation strategies.

OCEAN ACIDIFICATION IMPACT SWIMMING AND SETTLEMENT OF BUGLA NERITNA

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Increasing carbon dioxide emission will reduce surface ocean pH by 0.4 pH units by the end of this century. This process of ocean acidification is suggested to negatively impact marine organisms in processes such as growth, reproduction, settlement, and behavior. In particular, early planktonic stages are more vulnerable. However, to date, most studies focus on feeding larvae. Little is known about the effect of ocean acidification on lecithotrophic larvae.

Using *Bugula neritina*, a cosmopolitan bryozoan with lecithotrophic larvae, we investigated the effect of ocean acidification on this organism's crucial life-cycle transition. We exposed adults and their larvae to a control and a low pH (pH_T = 8.0 vs 7.6). We monitored the spawning, swimming behavior and settlement of the larvae and the offspring when they reached the juvenile stage. Our results show that the number of larvae released was not affected by lowered pH (48h exposure). Bryozoan's larvae swam 32% faster at low pH. Over a broader range of pH (8.0 down to 6.5), we observed that the larvae required more time to settle in low pH (7.6 vs 8.0). However, these acidified individuals had a faster growth rate after settlement. The observed acidification effects do not appear lethal, and may even be slightly positive, for the lecithotrophic *B. neritina*. And yet, prolonging the pelagic larval duration could increase the predation risk, alternated ecological interactions under climate change conditions should be considered in determining the population-level impact of ocean acidification.

POPULATION DYNAMICS AND ECOLOGICAL CHARACTERISTICS OF *NOCTILUCA SCINTILLANS* IN PORT SHELTER, HONG KONG: CASE STUDY

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Noctiluca scintillans is a red tide forming heterotrophic dinoflagellate, which is the most common HAB species in Hong Kong. The field study in Port Shelter during 4 October 2012 to 15 April 2013 showed that, *N. scintillans* occurred from 8 January 2013, and was generally higher on the surface layer, with a maximum abundance up to 1,976 cells L^{-1} at ST7 surface layer on 6 February. The abundance of *N. scintillans* was highly regulated by water temperature and potential food supply, as indicated by its strongly negative correlation with temperature and positive correlation with different sized Chl *a*, as well as major groups of microplankton, i.e. diatoms, dinoflatellates and ciliates. Some major groups of mesozooplankton kept up with the increase of *N. scintillans* abundance, indicating possible food competition among them. *N. scintillans* also occasionally fed upon tintinnids, copepods and copepods eggs, which might help it avoid starvation, simultaneously release part of the grazing pressure on phytoplankton and affect the recruitment of copepod. Comparison of the population growth of *N. scintillans* in the microcosms and real field situation revealed that, optimum hydrographical (temperature and water stability) and rich food supply were necessary for *N. scintillans* to seed and reproduce massively in Port Shelter, but its spatial patterns were then a result of biological and physical coupling, and mainly controlled by the physical accumulation process.

EFFECT TRIBUTYLTIN CHLORIDE (TBTCL) ON HATCHABILITY CYST OF ARTEMIASALINA

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Organotin compound are widely used in antifouling paints as bioactive agents against fouling organisms. In previously published reports, acute toxicity tests on *Artemiasalina* (Linnaeus, 1758) were only focused on a part of the life cycle of the *A. salina*. The aim of this study was to investigate the toxicities of tributyltin chloride (TBTCl) on the hatching stage of *A. salina*. According to the hatching test resulted more sensitive than acute mortality test to TBTCl. Significantly decreased the hatching percentage of *A. salina* cysts and prevented the hatching of larvae after expuser to different concentration of TBTCl. The effective concentration EC_{50} value for TBTCl was found as 50% effective concentration between TBTCl concentration and the cyst hatchability percentage was $(EC_{50} 46.48 \text{ ng.L}^{-1})$. In conclusion, toxicities of organotin compound should be tested not only on active nauplii but also at their hatching stage since TBTCl significantly decreased the hatching percentage of *A. salina* in the present study.

DISTRIBUTION AND SOURCE OF ORGANOCHLORINE PESTICIDES IN WATER OF QUANZHOU BAY OF FUJIAN PROVINCE, SOUTHEAST CHINA

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Sea water samples in the Quanzhou bay and fresh water samples from the river (both high and low flow seasons) to Bay were collected and analysed for organochlorine pesticides (OCPs, including HCHs, chlordanes and DDTs, total 19 organochlorine pesticides) by GC-ECD to study sources of the OCPs in the Quanzhou Bay of Fujian, China. In the high flow season, the concentrations of DDTs, HCHs and OCPs in the river water ranged from 0.95 ng·L⁻¹ to 9.09 ng·L⁻¹, 6.32 ng·L⁻¹ to 27.45 ng·L⁻¹ and 12.53 ng·L⁻¹ to 57.01 ng·L⁻¹, respectively. In the low flow season, the concentrations of DDTs, HCHs and OCPs in the river ranged from 7.79 ng·L⁻¹ to 12.7 ng·L⁻¹, 10.09 ng·L⁻¹ to 13.7 ng·L⁻¹ and 39.61 ng·L⁻¹ to 54.43 ng·L⁻¹, respectively. The ranges of DDTs, HCHs and OCPs in the sea water were from 0.79 ng·L⁻¹ to 6.51 ng·L⁻¹, 1.31 ng·L⁻¹ to 9.95 ng·L⁻¹ and 6.65 ng·L⁻¹ to 16.64 ng·L⁻¹, respectively.

The concentrations of the river water in the high flow season were higher than that in the low flow season. The variations of HCHs and DDTs concentrations in the high flow season were great than those the in low flow season. Concentrations of OCPs in the river water were significantly higher than that in the sea water, showing the flow of the river water into the Bay could be a source of the OCPs in the Quanzhou Bay. In the river water, α -HCH and γ -HCH were the dominant HCHs isomers in the high flow season, while α -HCH and δ -HCH were the dominant HCHs isomers in the low flow season. The ratios of α -HCH γ -HCH in the river water were all lower than 3, indicated that there was new input of lindane. In the sea water, α -HCH and γ -HCH were the main HCHs isomers, which was similar with that in the river water. It indicated that the river water is an important supply for OCPs in the sea water. The average ratio of α -HCH and γ -HCH in the sea water was 1.96, it also suggested there was new input of lindane. The ratios of (DDE+DDD)/DDT and *o*, *p*'-DDT/*p*, *p*'-DDT indicated that there may be newly pesticide application. And the ratios of DDE/DDD showed the breakdown condition in the river water was anaerobic, yet the condition in the sea water was aerobic.

INTERRELATION OF PERSISTENT ORGANIC POLLUTANT LEVELS AND TROPHIC LEVELS IN ANTARCTIC NOTOTHENIOID FISH AND INVERTEBRATES <u>F C Ko^{1,2}</u>, W L Pan¹, T H Chen^{1,2}, F W Kuo², S J Kao³, W H Wang⁴ and L S Fang⁵

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Notothenioid fish and invertebrate samples from Zhongshan Station, Antarctica were collected in the austral summers of 2009 and 2010. Samples from six species of fish and nine species of invertebrates were analyzed for persistent organic pollutants, including polycyclic aromatic hydrocarbons (PAHs), organochlorine pesticides (OCPs) and polybrominated diphenylethers (PBDEs), as well as d¹³C and d¹⁵N stable isotopes for trophic level determination. In Antarctic fishes and invertebrates, Σ_{23} PAH concentrations ranged from 6-27 ng/g ww and 7-753 ng/g ww, respectively, with phenanthrene at the highest concentration; Σ_{15} OCP concentrations ranged from 6-39 ng/g ww and 4-123 ng/g ww, respectively, with hexachlorocyclohexane (HCH) series as the main component, flowing were dichlorodiphenyltrichloroethane (DDT) series; Σ_{17} PBDE concentrations ranged from 0.1-0.4 ng/g ww and 0.1-125 ng/g ww, respectively, with BDE15 and BDE183 as the main component in fish, and varying components represented in different invertebrate species. The levels of PAH and OCPs in Antarctic organisms reached levels similar to those found in marine organisms from other regions of the world, while the levels of PBDE were more than 10 times lower than in marine organisms from other regions. POP concentrations in Antarctica organisms negatively correlated with trophic level, suggesting that specific biogeochemical processes were involved in the source, transport, exposure, and absorption for each group of contaminants and the biological variation among species, habitat interaction, diet and metabolism.

TOTAL MAXIMUM ALLOCATED LOAD OF PETROLEUM POLLUTIONS ON JURISDICTIONS IN BOHAI SEA BY LINKING LAND AND SEA

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With the rapid economic development of coastal and offshore oil exploitation, the risk of marine petroleum pollutions is unprecedented, which causes serious harm to the marine environment and ecosystems, has become a worldwide problem of marine pollution. More and more petroleum pollutions from Bohai rim, shipping and petroleum drilling platforms are discharged into Bohai Sea, and the petroleum pollutions concentration has exceeded seawater quality criterion seriously in Bohai Sea. The Total Maximum Allocated Loads (TMAL) management for petroleum is urgent to carry out. A method for calculation of TMAL of petroleum in Bohai sea based on jurisdictions by linking land and sea was established. TMAL of Petroleum pollutions for 51 Sea-sink Source Regions (SSRs), 356 counties and 6 petroleum drilling platforms around the Bohai sea were calculated. Results showed that the TMALs of petroleum pollutions in 51 SSRs for land sources and 6 petroleum drilling platforms for sea sources amount to 1.0×10^4 ton/year. The highest TMAL is the rivers, then the petroleum drilling platforms, and then the wastewater treatment facilities (WWTFs), about 0.22×10^3 , 0.13×10^3 and 0.11×10^3 ton/year on average, respectively. The TMAL of petroleum pollutions on source producing, source emitting and sea receipting are 4.7×10^4 ton/year, 1.5×10^4 ton/year and 1.0×10^4 ton/year in 356 jurisdictions, respectively. The TMAL is always higher in the areas of upstream and coast where water exchange quickly. Taking Laizhou Bay as an example, there are 19 jurisdictions in source producing petroleum pollutions and 27 jurisdictions in source emitting petroleum pollutions overload, 6 times higher than the average. The results can provide a scientific basis for the realization of "differentiated reduction" in Bohai sea.

PHOTOCATALYTIC DEGRADATION OF ORGANIC POLLUTANTS IN SEAWATER BY TITANATES

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The feasibility study for photocatalytic degradation of organic pollutants (methylene blue as the representative model compound) with titanate ion-sieves (Li₂TiO₃) that could also recover lithium from seawater was carried out in the present work. The titanate have the desired band gaps and UV/Vis light absorption capability for photocatalysis. The enriched A₂ ((Ti=O)O₄) and A₃ (TiO₆) photoactive sites on the titanates are also observed by the Gaussian-Lorentzian curve fitted pre-edge spectra of synchrotron X-ray absorption. The mean diameter (d) and polydispersity index (PI) of the lithium/protonated titanates in water and seawater were determined to determinate whether the photocatalysis is feasible during the recovery of lithium from seawater. After a 24-h UV-Vis light irradiation, the conversion of MB (1-X_{MB}) photocatalyzed by titanates is 0.76-0.93 approximately. However, in seawater with the greater mean diameter and polydispersity index of the lithium/protonated titanates, relatively low photocatalytic conversions (0.12-0.50) are found. It is also worth noting that the ion-sieve has desirable photoactive sites that photocatalytic degradation of organic pollutants can be achieved during the Li⁺-H⁺ exchange processes.

CADMIUM INDUCE APOPTOSIS IN MARINE CILIATE, EUPLOTES CRASSUS

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Heavy metals can cause genotoxic effects, such as oxidation of DNA indirectly by generating reactive oxygen species, leading to apoptosis of cell in living organisms. However, heavy metalmediated genotoxic mechanisms is not uncover in the ciliate. Here, apoptosis-related genes (*MKK7*, *MAPK2* and *AIF3*) were cloned and sequenced in marine ciliate *Euplotes crassus*. To measure extent of DNA damage, comet assay, and DNA fragmentation were performed, and transcriptional modulation of these genes and oxidative stress – related genes, *ogg1* was further analysed by using real-time RT-PCR after exposure to cadmium (Cd). As results, DNA fragmentation was not observed in this experiment condition, but DNA strand breaks was found by comet assay. The expression level of apoptosis-related genes were down-regulated in low concentration of Cd and then slightly up-regulated in high concentration, compared to control. *OGG1* gene expression level decreased significantly after exposure to Cd, indicating that Cd can induce DNA damage. These findings imply that Cd-mediated DNA damage can lead apoptosis in *E. crassus* by modulating the expression of upstream genes. This study would be helpful to understand molecular mechanisms of Cd– induced genotoxicity and apoptosis in ciliate.
CHARACTERIZING THE PAHS IN THE YANGTZE RIVER ESTUARY AND ADJACENT COASTAL ZONE: INFLUENCE OF ANTHROPOGENIC ACTIVITIES AND MARINE DYNAMICS

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77 sediment samples were collected from the Yangtze River Estuary (YRE) and its adjacent coastal zone (IACZ), to study the characterizations of polycyclic aromatic hydrocarbons (PAHs) and their influential factors such as anthropogenic activities and marine dynamics. We also calculated the cancer risk of sedimentary PAHs via ingestion and dermal contact using incremental lifetime cancer risk (ILCR) model. Total concentrations of the 16 priority PAHs (SPAHs) in the sediments from the 77 sites varied from 27.2 to 621.6 $ng \cdot g^{-1}$, dw (dry weight) with an average value of 160.1 $ng \cdot g^{-1}$, dw. Spatially, the Σ PAHs concentration presented a wide range of fluctuation and has an increasing tendency from north to south. In addition, there was a decreasing trend of the Σ PAHs values with the ascending distance from the estuary to the adjacent sea. In this study, the concentration of LMW PAHs was range from 2.5 to 185 ng \cdot g⁻¹dw, which account for 9.2%-69.6% of Σ PAHs with a mean value of 37.7%. In the comparison of LMW PAHs, the concentration of HMW PAHs was relatively high and ranged from 12.9 to 436.3 ng•g⁻¹dw, which account for 30.3%-89% of Σ PAHs with an average value of 62%. Marine dynamics were the mainly factors controlled the compositions and distribution of sedimentary PAHs in this area. PMF model was used to quantitatively evaluate sources of sedimentary PAHs, and four sources were identified: Factor 1 coal combustion (23.9%)/ Factor 2 petrogenic sources (23.6%) • Factor 3 wood combustion (11.5%) and Factor 4 traffic sources (40.9%). The results of PMF model shows anthropogenic activities were the mainly sources of PAHs in the study area. The level of cancer risk via dermal contact was higher than ingestion which at low level and only has low risk. The highest level of cancer risk occurred in the estuary and the outside of Hangzhou Bay, which even at high level ($\geq 10^{-3}$) and has high risk. This results indicated that the cancer risk via ingestion considered acceptable or inconsequential, however, the risk level via dermal contact is considered relatively serious, and some control measures and remedial actions should be conducted.

DEVELOPMENTAL TOXICITY OF SULFAMETHAZINE IN MARINE MEDAKA (ORYZIAS MELASTIGMA) EMBRYOS

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Bioaccumulation of Sulfamethazine (SMZ) in marine medaka (*Oryzias melastigma*) embryos, and its potential developmental toxicity were studied. Results showed that bioaccumulation of SMZ in embryos linearly increased with SMZ exposure concentrations and exposure time. Hemoglutination was found the main malformation in embryos stage, whereas yolk sac edema were mainly showed in larvae stage. The mortality and malformation rates of embryos and hatched larvae were induced by SMZ in a concentration-dependent manner. The best correlations between the embryonic mortality and hemoglutination rate might indicate that hemoglutination was probably the main cause of embryonic death. The embryonic heart beats increased with increasing SMZ concentrations on 4 dpf and 12dpf. Superoxide dismutase (SOD) and catalase (CAT) activities of embryos under SMZ exposure showed a significant dose-effect relationship. Furthermore, heart beat of *O. melastigma* embryos, SOD and CAT activities are so sensitive at the exposure concentrations of SMZ implied that they may be used as indicators of sublethal toxicity during the early development stage of *O. melastigma*.

DISTRIBUTION AND VARIATIONS OF POLYCYCLIC AROMATIC HYDROCARBONS AFTER OIL SPILL AT GUIMARAS ISLAND, PHILIPPINES

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On August 11, 2006, the Solar I tanker was hit by a hard rainstorm and sank off the coast of Guimaras Island in Philippines. About 350,000 tons of heavy fuel oil was spilled from the tanker, polluting about 200 km of the Guimaras shoreline, and seriously damaging coastal fisheries for several years. The spill also polluted over 450 ha of protected mangrove forests with complex topography. In general, the spilled oil compounds such as polycyclic aromatic hydrocarbons (PAHs) decrease with evaporation, diffusion, photooxidation, and biodegradation, and the time required the decrease should be shorter in tropical area than that in temperature temperate and boreal areas, because it's consistently high temperature and frequent storms cause in tropical area. However, little is known about the variations of composition and distribution of components derived from oil spill. In this study, we investigated these variations of distribution of parent PAHs and alkylated PAHs in sediments and aquatic organisms at Guimaras for up to 6 years after spill. PAHs and alkylated PAHs derive from oil were widely distributed in the shoreline of centring around south part of Guimaras island. PAHs and alkylated PAHs in shellfish collected from the rocky area were remarkably decreased one year after spill, because perhaps strong interferences with sea wave and frequent storm could cause and wash out. On the other hand, those in the area especially composed muddy sediments had been detected high concentrations for long period, because they could burrowed into sediments quickly after spill, and be trapped in the anaerobic condition. The trapped PAHs and alkylated PAHs slightly, but continuously dissolved in the water column, and had been accumulated in the aquatic organisms. These accumulations were especially serious in the organisms inhabited water surface such as oyster. The polluted conditions continued at the south and south east of island. For example, concentration of PAHs in shellfish at the south area was 295 ng/g wet weight at one month after spill, and 78 times concentrations observed in bivalves collected before PAHs rapidly declined to 85.6 ng/g at three months after the spill, and more slowly thereafter. the oil spill. After 13 months from spill, concentrations in shellfish were still elevated by a factor of 10 as compared to before the oil spill, suggesting that oil pollution was still continuing. Chrysene in parent PAHs and alkylated dibenzothiophene, phenanthrene/anthracene, pyrene/fluoranthene, and chrysene/benzo(a)anthracene in alkylated PAHs were abundant in shellfish at one month after oil spill, while phenanthrene and alkylated naphthalene were higher in spilled oil. These tendencies could depend on the metabolic ability in shellfish, and suggest that parent and alkylated PAHs of greater than 210 of molecular weight required more time for metabolism. In the investigation for 6 years, the composition of parent PAHs in shellfish changed from being dominated by higher molecular weight compounds such as chrysene and pyrene to lower molecular weight compounds such as naphthalene and phenanthrene. On the other hand, the composition of alkylated PAHs unchanged and alkylated pyrene/fluoranthene and chrysene/benzo(a)anthracene had been higher residue than others.

DISTRIBUTION OF NITROARENES IN SEDIMENTS AT OSAKA BAY, JAPAN

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Nitroarenes are nitro group on the parent polycyclic aromatic hydrocarbons, and mainly generate with the exhaust gas from the diesel vehicles. Some of nitroarenes has strong mutagenicity. The discharged nitroarenes in the atmosphere absorb on the suspended particles, and are finally expected to achieve water systems as river and sea with directly settling and/or rain. However, the polluted conditions by nitroarene are not less well understood in the aquatic environments, because they could exist in very low concentrations. In this study, we investigate the distributions of nitroarenes in sediments at 44 sites of Osaka Bay, Japan, closed to Osaka and Kobe cities with the high population densities. The sediment samples were collected by a research ship and humans in 2000 to 2006. The extracted fourteen nitroarenes in sediments were reduced to aminoarenes, and then they were acetylated. Acetylated targets were measured with LC/MS/MS. 1-Nitronaphthalene were detected from all sampling sites and the concentrations were from 7.91 to 786 pg/g dry weight. 1-Nitroprene, is generally, treated an indicator compounds in the nitroarene investigations, was detected in the most of sampling sites on shoreline, and was residue in high concentration especially at the closed-off section of bay, close to Osaka city. Perhaps, this tendencies could closely relate the heavy traffic in Osaka city, and in fact there are some busy highways near the sampling sites.

Total nitroarenes (nitroarenes) were distributed as following; that was detected especially high concentration at the closer sites to shoreline, while the concentrations tended to decline with increasing distance from shoreline. In addition, nitroarenes with high concentration distributed from Suminoe in Osaka prefecture to Amagasaki city in Hyogo prefecture. These sampling sites have some estuaries of rivers, and the nitroarenes carried with river water strongly contribute to their distributions at the closed-off section of Osaka Bay. There were a few sites with high concentrations of nitroarenes around the middle of Bay, and considerable nitroarenes could be settled into the Bay directly from the atmosphere. In addition, nitroarenes were also detected with high concentrations along with the east shorelines with industrial area. As a result, exhaust gas from industry was also one of important source to the pollution of nitroarenes in the coastal areas.

THE USE OF HOMING PIGEONS AS A BIOMONITOR FOR ATMOSPHERIC POPS IN GUANGZHOU

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With the worsening air quality in China, it is essential to monitor the contaminants of concern in the atmosphere, especially in the megacities. Traditional atmospheric monitoring, such as using passive air samplers provides the concentration data in gaseous phase and particulate phase, but lacks the consideration about bioavailability, not to mention assessing toxicity risk of long-time exposure. Instead, biomonitoring can make up for this. Homing pigeons raised in urban area are suitable biomonitors of air pollution in cities for three reasons: first, the homing ability makes homing pigeons live in their birth places during lifetime and keep exposing in air in the target area. Second, every homing pigeon has a foot ring recording its birth information, and usually they can live more than ten years. Last, more than 80,000 homing pigeon lofts are registered in China, spreading almost all cities in 34 provinces.

In this research, 29 homing pigeons in Guangzhou urban area were collected and grouped to 1, 5, 10 year age groups. After necropsy, histopathological assessment was conducted for lung and liver. Concentrations of polycyclic aromatic hydrocarbons (PAHs), organochlorine pesticides (OCPs), polychlorinated biphenyl (PCBs) and halogenated flame retardants (HFRs)in lung, liver and fat tissues were measured. There was positive correlation between the target contaminant concentrations and age of pigeons, indicating these persistent organicpollutants (POPs) were accumulated in the organisms through respiration. For the homing pigeons with high PAH concentrations in lung tissue, the pathological response was also observed. Female homing pigeons in 5 and 10 year groups have significantly lower concentrations of target POPs in fat tissue than the males, probably because the POPs could be transferred into eggs and eliminated from the body when the female lay. Compared with the data of atmospheric monitoring, biomonitioring data using homing pigeons provides a reference for assessing the respiration exposure risk of POPs to the residents in Guangzhou.

STUDY ON BIODEGRADABILITIES OF 3 NONSTEROIDAL ANTI-INFLAMMATORY DRUGS IN ZHUJIANG (PEARL RIVER) BASIN, SOUTH CHINA

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Newly emerged organic pollutants nonsteroidal anti-inflammatory drugs (NSAIDs) are ubiquitous in Zhujiang (Pearl River) basin, South China, and thus it is necessary to evaluate their environmental persistence (PBT/vPvB assessment) and ecotoxicological risks. Three most frequently detected NSAIDs, ibuprofen (IBP), acetaminophen (APAP) and diclofenac (DFC) were chosen as the target contaminants, and their ready biodegradability assessment were first conducted using the standard OECD tests (301B, 301D, 301F) according to the testing strategy of the OECD guidelines. Results showed that none of these three NSAIDs meet the strict definition of ready biodegradability (no less than 60% within 10-d window), even if APAP has the highest biodegradation rates in the 301B and 301F tests, which were 31.6% and 30.9% respectively. Moreover, in case of the negative results during the ready biodegradation measurements, inherent biodegradation tests (OECD 302C) as the second tier of OECD biodegradation testing were also conducted for IBP, APAP and DFC using two different respirometric systems (C.E.S® and OxiTop®) to determine their potential biodegradabilities under more optimized conditions. The comparison of C.E.S[®] and OxiTop[®] systems showed there is a clear tendency that OxiTop[®] gave lower amounts than C.E.S[®]. After 28 days incubation, up to 93.3% biodegradation of APAP was measured in C.E.S[®] while up to 33.7% in OxiTop[®]. Similar to the previous ready biodegradation tests, much lower biodegradation was observed for both IBP and DFC with 0% and 0% in OxiTop®, and 0% and 1.6% in C.E.S[®], respectively. The difference in biodegradation of IBP and DFC between these two systems was not so obvious. This might be due to the poor biodegradation of IBP and DFC in the present study. Findings of our study suggested that it's hardly for IBP, APAP and DFC to undergo rapid and ultimate biodegradation in the environment. APAP is much easier biodegradable than other two contaminants, indicating APAP will not be persistent and more attention should be paid to IBP and DFC for their potential toxicities and ecotoxicological risks in aquatic systems.

MICROPLASTIC INGESTION REDUCED ENERGY INTAKE IN THE CLAM ATACTODEA STRIATA BY FOOD DILUTION EFFECT

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The effects of microplastic concentrations (10 items l⁻¹ and 1000 items l⁻¹) on the physiological responses of *Atactodea striata*, including respiration rate, feeding rate and absorption efficiency, were investigated. The fates of microplastics ingested and the efficiency of depuration in removing ingested microplastics were also studied. *A. striata* could ingest microplastics and at the high concentration of microplastics, the clearance rate was reduced owing to the dilution effect of microplastics on the algal concentration. Both high and low concentrations of microplastics were found to have no effect on the respiration rate and absorption efficiency. As a result, microplastic ingestion would reduce the energy intake. Furthermore, the production of pseudofaeces reduced the amount of microplastics ingested and some microplastics were trapped and removed by faeces, resulting in a very small amount of microplastics stored in the body of the clam.

INFLUENCES OF SURFACE MODIFICATIONS OF ZINC OXIDE NANOPARTICLES ON THEIR TOXICITIES TO FRESHWATER AND MARINE MICROALGAE

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Zinc oxide nanoparticles (ZnO-NPs) are effective blockers of ultraviolet radiation but they can cause growth inhibition and mortality in various aquatic micro-organisms due to the release of zinc ions and the interaction between the nanoparticles and the cells. Recently, concerns have been raised over the potential environmental impacts of silane-coated ZnO-NPs because of their wide applications in commercial sunscreens and their easiness of being released into the aquatic environment. This study aimed to compare the physicochemical properties between silane-coated and uncoated ZnO-NPs, and elucidate their toxicities towards aquatic microalgae. The surface of ZnO-NPs (20 nm) was modified by 3-aminopropyltrimethoxysilane (A-ZnO-NPs), and dodecyltrichlorosilane (D-ZnO-NPs). These two coated-nanoparticles, uncoated ZnO-NPs and bulk ZnO were characterized in terms of particle size, zeta potential, aggregate size, dissolution and surface chemistry. Three freshwater algae and three marine algae species were exposed for 96 h to ZnO, uncoated ZnO-NPs, the two coated ZnO-NPs and ZnSO₄ at 10 concentrations ranging from 0.1 to 100 mg/L. The results showed that uncoated ZnO-NPs formed larger aggregates and released more zinc ions than the two coated ZnO-NPs. Although the sensitivity towards the test chemicals among the test algal species varied, A-ZnO-NPs and uncoated ZnO-NPs were consistently more toxic than D-ZnO-NPs in terms of algal growth inhibition. The marine diatom Thalassiosira pseudonana exposed to ZnO-NPs, A-ZnO-NPs and D-ZnO-NPs resulted in different gene expression profiles, suggesting that they exhibited different toxic mechanisms to this algal species. The results of the study provide some useful insights for development of eco-friendly nanoparticles for sunscreen products in the future.

METAL BIOACCUMULATION IN MARINE ZOOPLANKTON COLLECTED FROM THE COASTAL WATER OFF SOUTHWESTERN TAIWAN

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Three transects were established alone southwestern coast of Taiwan, transects from north to south were respectively extended from the Kaohsiung Harbour, Kaoping River estuary, and Fangshan River estuary. Three sites per transect were selected for sampling zooplankton and water on October 2015. Six metal including lead (Pb), cadmium (Cd), chromium (Cr), copper (Cu), zinc (Zn), and nickel (Ni) were analysed in the zooplankton using a flame atomic absorption spectrophotometry. Average metal concentrations in seawater were 0.56, 3.32, and 1.39 μ g L⁻¹ for Cu, Zn, and Ni, respectively. Metal concentrations of Pb, Cd, and Cr in water were lower than the method detection limits of those. Results showed the average of metal concentrations followed the hierarchy, Zn > Cu > Pb > Ni > Cr > Cd in zooplankton. The concentration metals of zooplankton shows variation in three transects. For all metals, the highest metal bioaccumulation was observed in the sites of Kaohsiung Harbour transect (49.7 μ g g⁻¹ for Pb, 6.3 μ g g⁻¹ for Cd, 10.7 μ g g⁻¹ for Cr, 115.4 μ g g⁻¹ for Cu, 354.6 μ g g⁻¹ for Zn, and 13.2 μ g g⁻¹ for Ni), which might be contributed by the industrial waste and sewage from Kaohsiung City. Zooplankton collected from transect of Kaoping River estuary revealed the relatively lower bioaccumulation of metals. Compared with the zooplankton in other regions, the concentrations of Pb and Zn in the present study were higher than those in other regions. The bioconcentration factor of zooplankton ranged within $10^3 - 10^5$ for all studied metal, and indicated that zooplankton in the seawater of southwestern Taiwan can accumulate metal even at background concentrations of metals.

ENZYMES AND LPO ACTIVITIES IN MARINE MUSSELS FOLLOWING *IN VIVO* AND *IN VITRO* EXPOSURE TO HEAVY METALS

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Severe pollution in the ocean has been found all over the worldand caused detrimental impacts to marine organisms. Heavy metals are typical contaminants with prooxidant effects which can generate reactive oxygen species (ROS) by catalyse Haber-Weiss and Fenton reactions. Marine mussel have been applied as good indicator in the marine ecosystem monitoring program due to its sessile filter feeding character and wide distribution. However, most of previous researchesfocused on in vivo responses in mussels. Theresearch achievements in our laboratory have showed accordant results of DNA damage in haemolymph caused by heavy metals both *in vivo* and *in vitro* exposure. And it took shorter examination period by in vitro experiment, suggesting it can be a quicker monitoring tool formarine ecosystem evaluation. It was designed to use mussels as model organisms to assess antioxidant response, especially the enzymes and lipid peroxidation(LPO)activities both in vivo and in *vitro*, induced byheavy metals. The possibility of using *in vitro* responses to indicated oxidative stress of environment will be assessed by analysing antioxidant related molecules activities, such as LPO contents, activities of glutathione peroxidase (GPx), superoxide dismutase(SOD) etc. In this study four heavy metals (Cu, Zn, Cd, Hg) at series concentrations were selected according to their relatively high distribution in coastal water. Tissues and cells from gills, digestive glands and haemolymph were dissected for the detection of tissue-specific antioxidant responses to different heavy metals. The result indicates that there are tissue-specific responses of anti-oxidative ability to heavy metals. Correlations of different enzymes activities can also be found between the enzymes activities in vivo and in vitro exposure, suggesting that it could be a better way to assess the environmental pollutants induced oxidative stress in the field. The same responding trend to particular heavy metal in the same tissue can also provide a clue about the antioxidative mechanisms in mussels.

LAND-BASED DISSOLVED ORGANIC NITROGEN DEGRADATION DYNAMIC AND BIOAVAILABILITY BY LINKING EXPERIMENT WITH MODEL IN BOHAI SEA

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In Bahai Sea, the concentration of dissolved organic nitrogen (DON) frequently exceeds that of dissolved inorganic nitrogen (DIN), including ammonium, nitrate, and nitrite. Recent evidence indicates that land-based organic N compounds are released into the DON pool and degraded from this pool by planktonic microbiota on timescales of hours to days. This observation suggests that many components of the DON pool can play an active role in supplying N nutrition directly or indirectly to phytoplankton and bacteria. Here we present a study of the degradation action of landbased DON in coastal water by linking experiment and model, focused mainly on dynamic and bioavailability. The results show that the degradation rate constant of DON from sewage treatment plant was faster than the other land-based sources, with significant differences (P<0.05), and the DON were classified into three group compounds according to the bioavailability. The degradation capacity might be affected by hydrodynamic facts, and it was higher on the conditions of higher stirring speed. The supplying of DIN pool from DON of Liao river, Hai river, Huang river, Xiaoqing river were discussed by a 3D hydrodynamic and water quality model of Bohai Sea, where there were more DIN supplied for DON of Liao river than the other rivers for the long flushing time in Liaodong Bay. There is a need for greater appreciation and understanding of the potential role of DON as a dynamic participant in the nitrogen cycle within coastal ecosystems.

GENOME-WIDE IDENTIFICATION OF ATP-BINDING CASSETTE (*ABC*) TRANSPORTERS AND CONSERVED FUNCTION OF PUTATIVE XENOBIOTIC TRANSPORTERS IN THE MONOGONONT ROTIFER (*BRACHIONUS KOREANUS*)

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The ATP-binding cassette (*ABC*) transporters are one of the largest gene families among animal taxa and known to involve in various biological processes with their transport ability of wide range of substrates across the membrane by using ATP-cleavage driven energy sources. In the genome of the rotifer *Brachionus koreanus*, 61 *ABC* transporters were identified and classified into eight distinct subfamilies (A-H) by phylogenetic analysis. *ABC* transporters in *B. koreanus* were consisted of 11 *ABCA* genes, 19 *ABCB* genes, 15 *ABCC* genes, 3 *ABCD* genes, 1 *ABCE* gene, 3 *ABCF* genes, 8 *ABCG* genes, and 2 *ABCH* genes. Of several subfamilies, extensive gene duplication and loss events were observed in synteny. Particularly, massive gene duplications in *P-gps*, multidrug resistance proteins (*MRPs*), and *Bk-Abcg-like* were observed. Their conserved functions in transport abilities as multixenobiotic resistance (MXR)-mediated ABC transporters were validated using specific fluorescence substrates/inhibitors. In this study, we provide the whole identification of *ABC* transporter superfamily that will be useful as the genomic resources for future toxicological studies with a better understanding of the comparative evolution of *ABC* transporter superfamily in invertebrates.

PRESENCE OF ENZYME GENES TO DIGEST CARBOHYDRATE IN CORAL GENOME AND THEIR POSSIBLE ROLES IN CORAL LIFE HISTORY

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Carbohydrate is one of the important components for maintaining life which exists in nature. Carbohydrate is the major energy source and the monosaccharide is an important material for supporting cell structures. Reef-building corals, which support the basis of coral reef ecosystem, are also thought to use carbohydrate for maintaining their life history. However, whether corals actually use carbohydrate has not been examined yet. Recently, genomic information of coral has expanded by next generation sequencing. We confirmed the presence of several kinds of enzyme genes which can digest carbohydrate such as cellulase and chitinase in coral genome (*Acroporadigitifera*). Thus, we tried to clarify the roles of these enzymes by focusing on gene expression patterns and their enzymatic activities.

We performed gene expression analysis as follows. Total RNA was extracted from five samples (gametes 15 h after fertilization, those 40 h after fertilization, planula larva, polyp, adult coral) by using TRIzol (Invitrogen), and these were treated with DNase I (Invitrogen) and cDNAs were prepared by using PrimeScript RT reagent kit (TaKaRa). The PCR primer for each gene was designed by including the intron based on genome information of A.digitiferaby using Primer3Plus. A total of 4 µl of each PCR product was used for 2% agarose gel electrophoresis to confirm the amplifications of a single band as visualized with gel red under ultraviolet light. We also performed cellulase and chitinase follows. Surface agarose plate assay as tissues of Pavonadivaricata, Pocilloporadamicornis, Montiporadigitata, Acroporadigitiferawere removed from eachorganisms by using water-pik and homogenized in acetate buffer (pH 5.5). Agarose plates containing each substrate were prepared. Each plate was incubated 3 days at 37°C. After that the plate was soaked with 0.1% Congo red, left for 1 h to stain and destained with 1 M NaCl.

As the results of gene expression analysis, we confirmed expression of cellulase-like and chitinaselike genes in some life stages of *A.digitifera*. As the results of agarose plate assay, we confirmed the activity of cellulase and chitinase enzymes in some corals species. Recently, coral can be treated based on the concept of holobiont (coral - symbiotic algae - bacteria). The activities of cellulase and chitinase were found in this study, but it is thought that bacteria around corals may show these activities. Thus, we need to perform DNA barcoding analysis to clarify the origin of enzymatic activities shown above in the future.

OXIDATIVES STRESS AND GENOTOXICITY BIOMARKER RESPONSES IN TILAPIA (*OREOCHROMIS NILOTICUS*) EXPOSED TO ENVIRONMENTAL CONCENTRATION OF 1-NITROPYRENE

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Combustion of petroleum products can form nitroarenes (NPAHs) such as 1-nitropyrene (1-NP). These pollutants are reported to be carcinogenic and can be found in all environmental compartments and accumulated by aquatic organisms. This study aimed to assess whether environmental 1-NP concentration will induce genotoxicity and oxidative stress in tilapia *Oreochromis niloticus*, a freshwater and estuarine fish. *O. niloticus* were exposed to waterborne 1-NP. Cellular antioxidant enzyme activity of glutathione peroxidase and oxidative damage, i.e., lipid peroxidation, protein and DNA oxidation were used as biomarkers of oxidative stress, while the micronucleus test was used for evaluation of chromosomal damage and was used as an indication of genotoxicity. In addition, 1-NP concentration in *O. niloticus* was measured using HPLC-MS/MS. Results showed that all biomarkers for oxidative stress positively responded and micronucleus and other nuclear abnormalities frequencies increased. This study showed that environmentally relevant 1-NP concentration in test water (0.15 ng/L) and in fish (3 ng/kg) induced genotoxicity and oxidative stress. In conclusion, exposure to lower waterborne 1-NP concentration can pose a risk to freshwater and estuarine organisms through accumulation.

GENOTOXICITY TESTING USING JAVA MEDAKA (ORYZIAS JAVANICUS)

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Genotoxic responses are often correlated with impairment of development, growth, and reproduction. Genotoxicity testing is an important part of the hazard assessment of chemicals. The present study attempts to assess the genotoxic response of Java medaka exposed to several pollutants by the use of micronucleus test. Nuclear abnormalities such as micronuclei are considered as well established indicators of cytotoxicity and genetic toxicology. Adult Java medaka was separately exposed to heavy metals (Cd, Hg, Cu, Zn) and glyphosate-based herbicide. Blood smear from the gills were stained with Giemsa and observed for micronucleus and nuclear abnormalities induction. A significantly higher (p<0.05) induction of micronucleus was found in the exposure to 10.0 ppb Cd and Hg compared to lower concentrations of Cd and Hg tested and compared to exposure to 10.0 and 100.0 ppb Cu and Zn. Zinc and Cu has no effect on micronucleus induction but Zn has higher potency in the induction of nuclear abnormalities induction. In exposure to glyphosate based herbicide, micronuclei induction started to occur in exposure to 30 ppm while nuclear abnormalities induction commenced in exposure to 10 ppm. Among the nuclear abnormalities scored during this study were notched nucleus, lobed nucleus, blebbed nucleus, vacuolated nucleus, binuclei and ringed nucleus. The genotoxic response exhibited by the fish is an example of short term bioassay that serves as a good example of how simple, fast and accurate results can be obtained through testing using this particular fish. Java medaka constitutes simple and effective models for evaluating and identifying the presence of genotoxic chemicals in the aquatic ecosystem.

Keywords: *Oryzias javanicus*, genotoxic, micronucleus, nuclear abnormalities, heavy metals, glyohosate.

GEOSPATIAL ZONING OF JIULONG ESTUARY BASED ON MULTIVARIATE ENVIRONMENTAL GRADIENTS

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It is important, but fundamentally difficult, to define a transitional waterbody and its role within a coastal system, as a means to both understanding and managing a river and its connected estuary. In this study, zonation of the Jiulong Estuary, China was attempted using a hierarchical classification approach, as defined in the European Water Framework Directive (WFD). Following the WFD the whole estuary is classified at two levels (discussed below), and this has resulted in the delineation of three sub-areas based on field investigations carried out during 2013-2014. The zonation approach considers where the transitional waterbody starts and ends and the relative merits of defining the estuary in terms of its biology, physics, chemistry, geographic nature and socio-economic units.

At the first level of zonation, the Jiulong Estuary is compartmentalised into a transitional sub-area and a coastal sub-area; on the second level of zonation, the transitional sub-area is further compartmentalised into two zones according to hydrological conditions, biological communities and their different responses to environmental stress. Furthermore, one-way Analysis of Variance (ANOVA) was adopted to test the viability of the zonation's that resulted from this process. By analysing the physico-chemical characteristics of water and sediments, as well as aquatic fauna communities, significant differences were found among all sub-areas' in terms of their specific characteristics. The results provide a spatial framework for local environmental management in order to assist in understanding processes and resultant patterns at the whole estuary level of analysis.

EXPOSURE TO BUTACHLOR CAUSES THYROID ENDOCRINE DISRUPTION AND PROMOTION OF METAMORPHOSIS IN *XENOPUS LAEVIS*

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Butachlor is extensively applied in rice paddy ecosystem in china, and has been widespread contaminant in the aquatic environment. Here, Xenopus laevis was used for the evaluation of teratogenesis developmental toxicity, and disruption of thyroid system when exposure to different concentrations of butachlor by window phase exposure. Acute toxicity investigation shown that 96 h-LC50 value of butachlor was 1.424 mg/L and 0.962 mg/L for tadpoles (starting from stages 46/47) and embryos (starting from stages 8/9), respectively. Exposure to butachlor caused malformation, including abnormal eye, pericardial edema, enlarged proctodaeum and bent tail. Window phase exposure test indicated that butachlor significantly promote the contents of wholebody thyroid hormones (THs, T3 and T4) at higher levels, indicating thyroid endocrine disruption. At 7 days, exposure to butachlor up-regulated the mRNA expression of genes involved in THs synthesis and metabolism (tsha, tg, tpo and dio1) and THs receptors (tra and trb). At 14 days, upregulation of the mRNA expression of genes related to THs synthesis and metabolism (tsha, tshb, tg, tpo, dio1, dio2 and ttr) and THs receptors (trb) were also observed after the exposure to butachlor. At 21 days, butachlor up-regulated the mRNA expression of tsha, tg, tpo genes and down-regulated the mRNA expression of tshb, tg, dio1, ttr and tra genes. These results showed that butachlor could change the mRNA expression of genes involved in the HPT axis and increase whole-body thyroid hormones levels of X. laevis tadpoles in a dose- and time-dependent manner, causing thyroid endocrine disruption and developmental toxicity.

MIXTURE OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS IMPAIRED THE OXIDATIVE STATUS OF ZEBRA FISH (DANIO RERIO)

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Non-steroidal anti-inflammatory drugs (NSAIDs) are considered emerging aquatic pollutants since they are commonly found in freshwater ecosystems in the high ng L^{-1} to low $\mu g \ L^{-1}$ range concentrations. Although the environmental occurrence of the most common analgesic and antipyretic compounds is well known, recently some investigations showed their potential toxicity toward non-target organisms. However, to date, limited information is provided about the joint toxicity of NSAIDs to aquatic invertebrates those in the environment are exposed to complex mixtures, which could lead to dissimilar adverse effects. The present study investigated the modifications of lipid peroxidation involved in the detoxification process and the responses of their associated enzymes activity, as well as whole-organism changes in zebra fish exposed to two NSAIDs diclofenac (DFC), acetaminophen (APAP), and their binary mixtures. Results showed that the growth, development and reproduction of zebra fish and the embryos were significantly affected during the 14-d exposures. As for antioxidative system, induction of glutathione Stransferase (GST), superoxide dismutase (SOD), and catalase (CAT) were observed in long-term exposure to DFC and APAP. Meanwhile, methane dicarboxylic aldehyde (MDA) content increased with the increasing NSAIDs concentrations and the delayed exposure time, displaying obvious dose-dependent and time-dependent patterns. Changes in SOD, CAT, and GSH activities likewise occurred with NSAIDs in isolated form and as binary mixtures. In summary, DFC, APAP and their binary mixtures significantly altered some physiological and biochemical parameters with detoxification metabolism in zebra fish, the integrated approach combining the response in molecule levels with the performance of the whole organism can help elucidate the toxic effects of NSAIDs and provide more insight into the mechanism of toxicity in aquatic organisms. Our results showed that mixtures of NSAIDs at low-level concentrations can impair the oxidative status of zebra fish, posing a serious hazard to the health status of such non-target organisms.

BIOEROSION OF CORAL SKELETAL BLOCKS IN HONG KONG: SPATIAL PATTERNS AND ENVIRONMENTAL INFLUENCES

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Bioerosion is an important force tipping the balance between reef accretion and destruction, but little is known about the rates and causing agents of coral bioerosion in Hong Kong. We deployed Porites skeletal blocks at 10 sites representing a gradient of nutrient enrichment and sedimentation in Hong Kong waters, and sediment traps to determine sediment and nutrient deposition rates. The skeletal blocks were retrieved after one year and scanned with microCT to determine the rate of bioerosion. The results showed that bioerosion rates varied greatly among the sites from 0.13 to 2.21 kg CaCO₃ m⁻² year⁻¹. There was substantial variation in the contribution of external bioerosion by sea urchins (0.0-25.66%) and internal bioerosion by boring bivalves (0.0-4.68%) and polychaetes (0.63-1.44%) at a given site. The external bioerosion rate was positively related to sea urchin density, and the polychaete bioerosion rate was positively related to sedimentation rate. However, the bivalve bioerosion rate had no clear correlation with sedimentation or nutrient deposition rate, due to the great various within and among sites. Overall, the rates of bioerosion are comparable with the range of bioerosion rates of coral substrates on the Great Barrier Reef. However, since Hong Kong corals grow much slower than tropical corals, our data at some of the most seriously eroded sites are alarming. The sea urchin *Diadema setosum* is an important external bioerosion at some sites in Hong Kong, consistent with recent reports of coral damage by this sea urchin. At some sites of high sedimentation rates, development of the internal borer populations was quick, perhaps because sedimentation is positively correlated with the abundance of planktonic food supply for the filter-feeing borers.

THE FUTURE OF INTEGRATED MULTI-TROPHIC AQUACULTURE IN THE SOUTH CHINA SEA

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Without seafood production from aquaculture humanity will exhaust all seafood resources. In the South China Sea open water aquaculture is popular but generates substantial waste from uneaten feed, faeces, dissolved excretory products and fish mortalities that are released directly to the surrounding environment. Detriments include changes to local ecology, algal blooms, and fish kills to wild and stock fish. Even at moderate scales these impacts can have economic and social implications that question the viability of aquaculture entirely. Implementing Integrated Multi-Trophic Aquaculture (IMTA) could offer a solution. IMTA systems mitigate environmental impact by reusing fish farm wastes as fertilizer and feed for commercially valuable species at lower trophic levels. The primary benefits are reduced environmental impact and improved economic stability. While some experimental IMTA systems do exist in the Asia-Pacific region, engineered IMTA has not been intentionally implemented at any large capacity. In Hong Kong, aquaculture in fish culture zones is almost exclusively fish monoculture. In this study a metric for waste is estimated by calculating the feed conversion ratio which can be used to determine the amount of feed not converted to fish biomass. The environmental, economic, and social scope for IMTA is identified.

ANTIFOULING EFFICACY OF A CONTROLLED DEPLETION PAINT FORMULATION WITH ACETOPHENONE

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Biofouling is an inevitable problem continuously occurring on marine fishing vessels and other small crafts. The nature of antifouling (AF) coatings used to prevent the biofouling on these small vessels has great environmental concern. Therefore, efficacy of a nontoxic AF candidate, acetophenone was evaluated in preliminary laboratory assays using marine bacteria, diatom and *Ulva* spores. At a low concentration of 100 μ g cm⁻² of acetophenone coatings, spore attachment of a green fouling alga was significantly reduced (p < 0.01). Similarly acetophenone coatings at 40% level significantly inhibited the diatom attachment. Further, this new nontoxic AF agent acetophenone was incorporated into controlled depletion paint (CDP) and a field study was carried out at Ayajin harbor, east coast of Korea. The performance of newly formulated coatings was compared with control (base paint) and reference coatings (TBT & copper-based). Fouling coverage (%), biomass and fouling resistance (%) were estimated. On CDP coatings made with acetophenone (40%), significant decrease in fouling biomass was estimated (p < 0.01). On acetophenone-CDP coatings fouling of a green alga, Ulva pertusa was found to be less, whereas acetophenone-CDP coatings prepared with the booster biocide (zinc pyrithione) were very effective on animal foulers and their AF efficiency can be comparable to copper-based commercial formulations.

PASIG RIVER UNIFIED MONITORING SYSTEM (PRUMS) OF PASIG RIVER WATERSHED, METRO MANILA, PHILIPPINES

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The Pasig River is a major river that drains the tidally-influenced Pasig River watershed of the Metropolitan Manila, Philippines and connects the inland Laguna Lake and the Manila Bay. For decades, the Pasig River had been a pollution hotspot that by virtue of Executive Order 54 series of 1999, the Pasig River Rehabilitation Commission (PRRC) was created and mandated to ensure that the waterway is rehabilitated to its historically pristine condition conducive to transport, recreation and tourism as described by Water Quality Class C Standard. The Pasig River Unified Monitoring System (PRUMS) was established in 2009 by the Pasig River Rehabilitation Commission to monthly monitor 14 stations along the Pasig River and selected major tributaries based on the commonalities in water sampling points along the river by different government agencies. The water quality parameters monitored were based on the D.A.O. 34 series of 1990 water quality Class C standard. The annual average results of dissolved oxygen measurements show hypoxic conditions. The measured BOD fail the <10 mg/L standard with spatial and temporal variation. San Juan River, a major tributary, contributes the highest BOD load with annual average of 102.97 \pm 22.86 mg/L BOD in 2015 compared to the 37.26 \pm 22.79 mg/L 2009 baseline data. Generally, the monitored stations pass the <10 mg/L standard for nitrate but fails the <0.4 mg/L standard for phosphate. The PRUMS project reveal no significant improvement in the water quality of Pasig River in terms the dissolved oxygen, biochemical oxygen demand, nitrate and phosphate.

CONTAMINATION OF ORGANOCHLORINE PESTICIDES IN SURFACE SEDIMENTS OF THE BERING SEA, CHUKCHI SEA AND ADJACENT ARCTIC AREAS

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The contamination of organochlorine pesticides (OCPs) were investigated in the surface sediments from Bering Sea, Chukchi Sea and adjacent Arctic areas during Fourth Chinese National Arctic Research Expedition (4th CHINARE-Arctic) in 2010. Fifteen kinds of OCPs were detected in these samples including HCB, DDT, HCHs, Trans/cis-chlordane, endosulfan etc. The most abundant OCPs were HCB with the average concentrations of 3564.53 \pm 2488.24 and 2414.02 \pm 1595.03 pg/g (d.w.) in Bering Sea, Chukchi Sea and Arctic area, respectively. The average concentrations of HCHs (sum of α , β , γ , and δ isomers) showed no obvious latitudinal difference, with the close similar concentrations in the Bering Sea (4066.55 \pm 3697.70 pg/g d.w.) and Chukchi Sea and adjacent Arctic areas (3813.39 \pm 2602.75 pg/g d.w.). However, γ - HCH and o,p'-DDE were dominant in Bering Sea, α -HCH and p,p'-DDD were dominant in Chukchi Sea and adjacent Arctic ocean. DDT/DDTs >0.5 in Bering Sea indicated the continuous input from the urbanized and industrial region of Northern American. DDTs in surface sediments increased in Chukchi Sea (4669.28 \pm 1643.54 pg/g d.w.) with the latitude to indicate the long-range atmospheric transport. Comparing with other coastal environments, the results indicated OCPs residue were still low in surface sediments of Bering, Chukchi Seas and adjacent Arctic areas.

This work was supported by National Natural Science Foundation of China (NSFC) Project. (41276066).

ALGAE GROWTH PROMOTING METABOLITES PRODUCED BY AN ALGAL-BACTERIAL CONSORTIUM DURING THE DEGRADATION OF PYRENE

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A wide variety of microorganisms, including bacteria, fungi and algae are capable of polycyclic aromatic hydrocarbons (PAHs) degradation. A consortium constructed by PAH degrading microalga (*Selenastrum capricornutum*) and bacterium (*Mycobacterium* sp. strain A1-PYR) were reported could accelerate pyrene degradation. However how they interact with each other is still unknown for us.

The effects of pyrene and pyrene metabolites on algal cell division in the consortium were investigated in the present study. In the mixing culture of algae, bacteria and pyrene, the growth rate of algae was obviously increased from day 7 onward. The cell size of algae in the mix culture were smaller than the control (without pyrene and bacteria), though the chlorophyll contents of algae were not significant different between the two groups. In day 10, the cell cycle analyses of algae and the the concentration of pyrene metabolites in culture medium were detected. The increasing S phase fraction of cells and the decreasing percentage of G2/M phase fraction of cells shortened the cell cycle. Meanwhile, the pyrene metabolites kept on accumulating.

The results showed that pyrene metabolites produced by the consortium might shorten the cell cycle of algae, so that stimulate the growth of algae and heightened the metabolism levels of cells.

SOURCES, DISTRIBUTION AND MIGRATION OF MERCURY IN CHINESE COASTAL MARINE SEDIMENTS

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China is the country with the largest usage and emission of Hg^[1], and is therefore confronted with much pressure in dealing with Hg pollution. Marine ecosystem is an essential component in global mercury (Hg) cycling^[2, 3], and coastal regions act as a dynamic link between terrestrial and marine ecosystems, with sediments receiving Hg from both natural and anthropogenic sources. Since China has broad coastal regions and cities around coastal regions are mostly more developed and industrialized than western inland regions, it is necessary to study the Hg pollution status in Chinese coastal sediments. Recently, the use of MC-ICP-MS for high precision analysis of the seven natural isotopes of Hg has enhanced our understanding of both sources and biogeochemical processes. Determination of mass-dependent (MDF) and mass-independent (MIF) fractionation patterns provides useful information in tracing sources and environmental processes of Hg in sediments.

This work studied the sources, distribution and migration of Hg in Chinese coastal marine sediments by comprehensively analyzing total Hg (THg), stable Hg isotopic ratios, total organic carbon (TOC), and pH in 220 surface sediments from Chinese marginal seas, together with further analyzing the sediment particle size distribution and ocean current circulation. Large spatial variations in THg (from 7.0 to 159.6 μ g kg⁻¹), δ^{202} Hg (from -2.43 to -0.09‰) and Δ^{199} Hg (from -0.08 to +0.31‰) were observed in the sediments. Spatial distribution of THg displayed a general offshore decreasing trend towards the outer continental shelf. An isotopic triple-mixing model based on δ^{202} Hg and Δ^{199} Hg identified four primary Hg sources: industrial Hg, watershed/ urban Hg, precipitation and continental background. The results showed that industrial Hg sources dominate in Bohai Sea sediments, while precipitation sources dominate in Yellow Sea sediments. For East China Sea, the coastal and estuarine regions are dominated by both industrial and watershed/urban Hg sources, while the offshore open sea regions are dominated by precipitation sources. Ocean currents played a key role in the migration of Hg to the open sea, and sediment particle size affected the re-distribution of Hg, with mud deposits containing higher percentage of TOC as the main sinks of Hg. The Δ^{199} Hg/ Δ^{201} Hg ratio of ~1 indicates that a fraction of Hg has undergone photo-reduction process.

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APPLICATION OF BIOSTIMULANT BALL DEPTH EFFECT IN CONTAMINATED COASTAL SEDIMENT TO EVALUATE THE PAHs DEGRADATION AND ENHANCE THE BACTERIAL COMMUNITY USING PYROSEQUENCING

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Aim of the study s to analysis the biostimulant ball depth effect and to find out the optimum depth in contaminated coastal sediment. 16 types of two-ring, three-ring and four-ring poly aromatic hydrocarbons (PAHs) examined in this study. Furthermore, Microbial diversity was evaluated using 454 pyrosequencing and analyses PAH degrading sulphate reducing bacteria (SRB) in the sediment. Twelve contaminated sediment columns were prepared along with blank and each column containing biostimulant ball without blank. The BSB depth was varied from 0, 3, 6 and 10cm as well as month varied from 0 to 6 month. BSB is a biostimulant agent containing acetate, nitrate and sulphate which supply the nutrients for SRB to enhance the activity and it can provide electron donor and electron acceptor for the SRB to degrade 16 types of PAHs. Maximum degradation of PAHs were observed from 0cm and 3 cm and degrade efficiencies were ranging from 77% to 100%. Napthalene, Benzo (a)anthracene, Chrysene were degraded up to 100% in BSB added 0cm and 3 cm depth. 5 ring compound of Benzo (b) fluoranthene, Benzo (k) fluoranthene, Benzo (a) pyrene and Dibenzo (a,h) were degraded in the order of 93.7%, 94%, 93.5% and 94% respectively and 6 ring PAH of indole (1, 2, 3- Cr) was reduced 62% and also PAHs degrading SRBs are Desulfobulbaceae, Desulfobacteraceae, Desulfofustis, Desulforhopalus, Desulfobacterium, Desulfosarcina, Desulfopila, desulfococcus and Desulforhopalus. While compared to 0cm and 3cm depth, 6cm and 10cm depth had less effect to degrading PAHs. This result found that BSB has effective to enhance the activity of SRB species to degraded PAHs from the contaminated coastal sediment.

EVALUATION OF SEDIMENT COLLECTED FROM TOKYO BAY WITH EMBRYOS OF JAVA MEDAKA (*ORYZIAS JAVANICUS*)

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Because of the low water solubility, hydrophobic chemicals widely spread in the aquatic environment, and then continuously settle on sediments. They could become one of threats to the aquatic organisms. Especially, sediments of coastal area near the city with high density of populations and industrial area are polluted by chemicals. Sediments are also important place not only for the demersal fish but also fish spawned sedimentation eggs. Therefore, the investigations for sediments toxicity to aquatic organisms are important.

Java medaka (*Oryzias javanicus*) has been increasing to use in several ecotoxicological studies. In this study, we used the embryo of Java medaka to evaluate the toxicities of sediments collected from Tokyo Bay, Japan. Embryo as fish early life stage assay are particularly suitable for chemical testing, because they are particularly sensitive to chemicals, easy to observe their abnormalities, and no-required the large scale facility. However, the evaluation of field sediment toxicities polluted the various chemicals have not been performed with fish embryo test.

We collected the sediment samples from seven sites (st.1 to st.7) in Tokyo Bay, Japan, where closes to the overpopulated and industrial areas, and evaluated the sediment toxicities using embryo of Java medaka. Polycyclic aromatic hydrocarbons (PAHs) and heavy metals (Cd, Cu, Pb and Zn) in their sediments were determined by GC/MS and Atomic Absorption Spectrometry. Total carbon and nitrogen were determined by a CHN analyzer. While no mortality was observed in control (silica sand), the mortalities of embryos some reared with sediments collected from the sites close to industrial area achieved around 50%. Their delay of hatching rates were also observed, and they could cause by the mixed exposures of several chemicals, because their total carbons were higher than that in other sites, and chemicals could be residue in high concentrations absorbed to carbon in their sediments. Some abnormal embryos with edema, scoliosis, and incomplete development in some parts, were observed in larvae exposed to sediment collected from Kawasaki city, Kanagawa Prefecture. These abnormalities could cause from the high concentration of PAHs, because high concentrations of PAHs were detected from the site. Our method to evaluate the sediment toxicities can efficiently detect the effects derived from the chemicals in the field sediments and must become powerful tool as a risk assessment of environmental sediments.

BACKGROUND LEVELS OF GLYPHOSATE IN THE FORAGING AREAS OF THE ENDANGERED MILKY STORK POPULATION IN MALAYSIA

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Glyphosate is the most widely used herbicide in the world since its first introduction in the 1970s. About 15 million liters of glyphosate-based herbicides were sold in Malaysia annually and the number is expected to increase. Oil palm plantation is one of the most important industry the utilized this herbicide. Although its accumulation and direct impact to top predators are unlikely to occur, it still has the potential to affect the lower trophic community, causing imbalance to the local food web. Kuala Gula Bird Sanctuary is home to several endangered species like the Milky stork population (Mycteria cinerea). It also serves as an important stopover site for thousands of migratory birds during their migration. Considering the presence of extensive palm oil plantations in the area, there is a need to assess the glyphosate levels in the aquatic environment of Kuala Gula. Sediment samples were taken from areas frequently visited by the Milky stork population. The herbicide was by HPLC-UV detection. with previous derivatization 9analyzed using fluorenylmethylchloroformate (FMOC-Cl). Glyphosate levels in the sediments ranges from 0.26 -1.72 mg/kg. The spatial variation of glyphosate levels are linked to the distance of the source i.e. palm oil plantation and application time of the herbicide in the area. The findings also serve as baseline data for future study in the area.

Keywords: Glyphosate, Kuala Gula Bird Sanctuary, Milky stork, sediments, Malaysia

APOSYMBIOTIC PRIMARY CORAL POLYP COUNTERACTS ACIDIFICATION BY ACTIVE pH REGULATION

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Stony coral skeletons are critically important biological structures that support huge areas of tropical and subtropical marine life and provide natural barriers against waves caused by storms. However, the mechanism of coral calcification and how it is affected by ocean acidification remain unknown. Therefore, to address these issue, we performed direct pH imaging at calcification sites (subcalicoblastic medium, SCM) to highlight active pH up-regulation in live aposymbiotic primary coral polyps when treated with acidified seawater. Alkalization and acid-induced oscillations in pH in the SCM (pH_{SCM}) and occasional acid-induced pH up-regulation waves, which propagated among the SCMs after exposure to acidified seawater, were observed for the first time by an innovative vital staining method. We demonstrated that corals can regulate pH_{SCM} more actively than was previously believed. We believe that corals sense the ambient seawater pH via their inherent pH sensitive systems and regulate pH_{SCM} through several types of pH-regulating ion transporters that coordinate with multicellular signaling occurring within the coral tissue.

REDUCTION OF CHLORINATION BY-PRODUCT FORMATION IN A COASTAL POWER PLANT

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In coastal power plants where seawater is used as a coolant, chlorination is generally practiced to overcome the problem arising due to the biofouling, which occurs due to the presence of micro and macro organisms in the seawater. Though the control of biofouling can be achieved by many means, addition of chlorine as a biocide is prevalent due to its broad-spectrum activity. However, in addition to its role as a biocide, chlorine also poses a health hazard by producing chlorination by-products such as trihalomethanes (THMs), which have been reported to be carcinogenic. Hence, the removal of THMs post chlorination remains as important as the control of biofouling. Though there are various methods established for the removal of THMs from drinking water sources, there are very few methods available for the removal of the same from seawater which is used as a coolant in power plants and is ultimately discharged into the open sea. With the objective of the removal of the THMs from the seawater, a few studies have been carried out using a mesocosm facility to find out the influence of natural processes like evaporation. Moreover, experiments were also carried out to find out the influence of temperature in the removal of THM from seawater. In the mesocosm experiment seawater was dosed with 2.1 mg/L of chlorine to produce a residual of 0.2 mg/L after fulfilling the demand of 1.9 mg/L which was measured at the beginning of the experiment. The results showed that the THM concentration reduced slowly from 108.5µg/L to 85.8µg/L in 420 minutes; it took about 18 days for the THM levels to decrease to below detectable limit. In the experiment conducted to study the influence of temperature on THM removal, the percentage reduction of THM from 30°C to 40°C was compared with the THM formed at room temperature (28°C). At 30°C, the percentage reduction was 1.72, at 34°C it was 15.1, at 38°C it was 26.8 and at 40°C the reduction of THM was 31.0%. Through this study it is established that THM removal increases withincreasing rate of evaporation and temperature, due to the volatile nature of the compounds. The paper discusses the possibility of THM reduction in power plant effluents.

SEPARATION AND CHARACTERIZATION OF HEAVY METALS FROM HARBOR SEDIMENTS

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Contaminated sediments in the harbors remain being a significant issue during dredging operations. Numerous methods have been suggested in recent years for remove, treating and beneficial uses of harbor sediments. In this research, the basic characteristics and the geochemistry of metals in the Kaohsiung harbour sediments were studied, and which were subjected to pH-dependent leaching test and column test. The results of experimental leaching tests of harbor sediments are compared with computer calculations. This computer program allows to predict the metal concentrations and the pH of leaching solution at equilibrium, along with the minerals that precipitate and the organic characteristics react with the metal ions forming into stable organic metal compounds.

The result of laboratory research showed that the content of heavy metals contaminants such as Zn, Cu, Ni, Pb, Cr exceeded the sediment standards in Taiwan. Results from the chemical washing technique with HCl, H₂SO₄, HNO₃ as washing reagents showed that heavy metals could be removed in the order Zn 84% > Ni 72% > Pb 57% > Cu 45% > Cr 43% when applying 1N HCl as reagent. The pH-dependent leaching test confirmed the high leachability of Zn in lower pH environment. Results from the chemical extraction technique using Ethylenediaminetetraacetic acid (EDTA) and citric acid (CA) as reagents showed that heavy metals could be removed in the order Zn 78% > Ni 48% > Cr 28% > Pb 20% > Cu 2% with 0.1M CA as reagent. And it is worth notice that the Cr could only be extracted by 0.1M CA, it would not react with EDTA.

ALLELOPATHIC EFFECTS OF LEAF LITTER LEACHATES FROM *KANDELIA OBOVATA* AND THEIR PURIFIED CONDENSED TANNINS ON GERMINATION AND GROWTH OF *AEGICERAS CORNICULATUM*

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Kandelia obovata (Ko) and Aegiceras corniculatum (Ac) are common and dominant plant species in mangrove swamps in South China, and distribute in the same tidal zones along the coastline. However, how one species interacts with the other on its germination and growth is seldom reported. The present study aimed to determine the allelopathic effects of leaf litter leachates (LLLs) from Ko and their purified condensed tannins (PCTs) on the germination of propagule and growth of Ac by pot experiments. Replicate pots containing five different levels of LLLs (0, 2, 10, 20 and 50 g L^{-1}) of leaf litter in artificial seawater at a salinity of 10 parts per thousands (ppt) and PCTs (0, 10, 100, 200 and 600 mg L⁻¹) of purified tannins in 10 ppt artificial seawater were separately prepared and propagules of Ac were placed in each treatment. At the end of 3-month experiment, initiations of shoot and root during propagule germination were inhibited in 20 and 50 g L⁻¹ LLTs, respectively. The growth indicators, including the biomass of root, stem and leaf, length of root and stem, and number of root and leaf, also showed changes with different levels of LLLs but the responses were different among these indicators. The effects of PCT treatments were similar to that of LLLs. The germination of Ac propagule decreased gradually with increasing concentrations of PCTs, especially when the levels were $\geq 200 \text{ mg L}^{-1}$. On the other hand, the growth of Ac seedlings decreased in higher concentration (200 mg L⁻¹) but increased in lower concentration (10 mg L⁻¹) of PCTs. These results indicated that the leachates from the leaf litter of Ko, in particular, their purified condensed tannins, exerted an inhibition on propagule germination and seedling growth of Ac, and the inhibitory effects were concentration dependent. The present study suggested that allelopathy of condensed tannins from leaf litter might be one of the driving forces that could regulate the regeneration of a mangrove forest.

POPULATION DYNAMICS OF THE ROCK SHELL *REISHIA CLAVIGERA* ASSOCIATED WITH DIFFERENT DEGREES OF ORGANOTIN CONTAMINATION IN HONG KONG

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The application of organotin compounds (OTs) as antifouling paints on ship hulls and open-water mariculture cages has been partially banned in Hong Kong since 1992, and globally prohibited by the International Maritime Organization since 2008. It is, therefore, anticipated that there is a recovery not only on imposex-affected neogastropod individuals but also on their populations worldwide. A 25-month population dynamics study on the rock shell *Reishia clavigera* was comprehensively conducted in six locations around the coastal waters of Hong Kong covering different degrees of OT contamination. The abundance and size of *R. clavigera* over a 20-m transect in mid and low shores were monitored bimonthly. We found that there were higher abundance and density of rock shells in clean sites, while the animals tended to grow faster in polluted sites. Over time, recruitment of this species was found in clean sites while in polluted sites such recruitment was limited. The results suggested no apparent recovery of the local *R. clavigera* populations probably due to the fact that OT contamination, especially triphenyltin, is still prevalent in this region. This study, therefore, calls for immediate mitigation and long-term monitoring of OT contamination in the marine environments of Hong Kong and South China.

THE SPATIAL PATTERN OF THE BENTHIC MOLLUSCA DRIVEN BY PEARL RIVER INPUT AND ANTHROPOGENIC POLLUTION IN HONG KONG WATER

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Hong Kong is situated at the mouth of the Pearl River Delta. The annual freshwater input to the western part of Hong Kong shapes the hydrography of Hong Kong coastal water especially in the wet season from June to September. Salinity gradient is established due to high volume of Pearl River outflow from lower salinity at the western water and higher salinity at the eastern water. This salinity gradient with occasional eutrophication and anthropogenic pollution created highly diverse habitats for the sub-tidal organism in Hong Kong water. Mollusca, as the largest marine phylum and most of the time with relatively low trophic level, is an important group to study for assessing the ecosystem health. The spatial pattern of molluscs in Hong Kong sub-tidal water was described in this study. 229 species of molluscs from 69 families were recorded by the bottom trawl survey from 2012 to 2016. The molluscs community in western, eastern and southern sub-tidal water differed significantly. Highest biomass and abundance were observed in western water, while highest number of species was found in southern water. Nassarius siguijorensis, a scavenger, was found in all the study sites in a high abundance possibly due to their high tolerance to salinity and abundance of food supply caused by anthropogenic pollution. Turritella bacillum, a suspension feeder, was found to be the most dominant molluscs' species in the eastern and western water which were generally considered as a much polluted area when comparing to the southern water. The nutrient-rich fresh water input from Pearl River and sewage discharge to Tolo channel from fish farms and the town may explain why the Turritella bacillum was so abundant in those area. This study provides not only important information for future Mollusca study but also explores the possibility to use *Turritella bacillum* as an indicator for pollution for better management purpose.

APPLICATION OF IN SILICO TECHNIQUE IN ECOLOGICAL RISK ASSESSMENT OF MAIN SPILLED OIL TOXICITY COMPONENTS

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Polycyclic aromatic hydrocarbons (PAHs), a class of ubiquitous pollutants in marine environments, exhibit moderate to high adverse effects on aquatic lives and humans. However, the lack of toxicity data of PAHs to aquatic organism limited the evaluation of their ecological risks. In silico technique such as quantitative structure-activity relationship (QSAR) computational models provide a new idea for filling the toxicity data gap and satisfying the need of ecological risk assessment. In the present study, aquatic predicted no effect concentrations (PNECs) of 16 priority PAHs were derived and their probabilistic ecological risks in seawater of Liaodong Bay, Bohai Sea were assessed. QSAR method was adopted to achieve the predicted chronic toxicity data for the PNEC derivation. Good agreement for aquatic PNECs of 8 PAHs based on predicted and experimental chronic toxicity data was observed ($R^2 = 0.746$) and the calculated PNECs ranged from 0.011 to 205.3 µg/L. A significant log-linear relationship also exists between octanol/water partition coefficient and PNEC derived from experimental toxicity data ($R^2 = 0.757$). Similar order of ecological risks for the 16 PAH species in seawater of Liaodong Bay was found by probabilistic risk quotient and joint probability curve methods. The individual high ecological risk of benzo(a)pyrene, benzo(b)fluoranthene and benz(a)anthracene needs to be focused. The combined ecological risk of PAHs in seawater of Liaodong Bay calculated by joint probability curve method was 13.9% indicating a high risk caused by co-exposure of PAHs. The sources of combined ecological risk uncertainty for Σ PAHs in the probabilistic risk quotient method mainly come from exposure concentration data (12.7%) and the toxicity data (87.3%) by Monte Carlo simulation sensitivity analysis.

POTENTIAL ECOLOGICAL AND HUMAN HEALTH RISK ASSESSMENT OF HEAVY METALS IN PM₁₀ OF ROAD DEPOSITED SEDIMENTS (RDS) IN THE MAJOR INDUSTRIAL AREAS ALONG THE COAST OF SOUTH KOREA

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Resuspension of road deposited sediments (RDS) is one of the major sources of atmospheric particulate in industrial area environment. Particle below 10 µm diameter (PM₁₀) in RDS (PM₁₀) RDS) can be a reservoir of large amount of hazardous pollutants and resuspension of those PM₁₀ RDS can be important carrier of pollutants to nearby estuarine environments with long-rage transport. The aim of this study is to investigate the potential ecological risk of metals in PM₁₀ RDS in the major industrial areas along the coast of South Korea. PM₁₀ fractions were directly separated from total RDS collected at 165 sampling sites in the 9 large industrial areas nearby estuarine in December 2014. The mass concentration of PM₁₀ RDS samples ranged from n.d to 470719 (8555±7722) mg/m². The mean values of heavy metals were 893, 258, 1223, 7501, 194, 29.6, 2539, and 2.39 for Cr, Ni, Cu, Zn, As, Cd, Pb, and Hg, respectively. Cu, Zn and Pb, vehicle-related pollutants, showed the highest levels in PM₁₀RDS from Onsan industrial complex. We also tested PM₁₀ RDS ingestion by rotifer *Brachionus sp.* to study potential risk of PM₁₀ RDS in estuarine environment. PM₁₀ RDS were observed in the gut of tested adult Brachionus sp., implying that hazardous heavy metals with high concentrations in PM₁₀ RDS can cause adverse effect to *Brachionus sp.* and, by extension, the marine environment. Resuspended PM_{10} RDS can potentially give hazardous effect to residents and workers living in these area.
HEAVY METALS IN ROAD DEPOSITED SEDIMENTS IN A BEACH TOWN IN BUSAN, KOREA: DISTRIBUTION AND POTENTIAL IMPLICATION FOR COASTAR POLLUTION

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The aim of this study was to investigate distribution and potential implication of heavy metals in road deposited sediments (RDS) in beach town of Busan Korea. 9 RDS samples were collected from the kerbs of traffic roads, and heavy metals in RDS samples were assessed. The contamination levels in RDS from study area were relatively similar or higher than that of other polluted cities. Zn, Ci, Cr and Pb, vehicle-related pollutants, were higher than that of other elements, such as Li, Co, Ni, As and Cd in RDS. Zn was most enriched element followed by Cd and Pb. To predict input of eluted heavy metals from RDS by rain water, we tested artificial rain fall simulations with RDS collected from these study areas. The most extractable elements, by artificial rainwater, were Cu, Zn and Cr among the heavy metals in RDS, indicating that those metals can be pollution source of the adjacent estuarine environment. RDS in urban areas can be directly transported to coastal environment with storm water which has been recognized as a major contributor of pollutants to coastal waters nearby urban areas. Therefore, seawater soluble fractions of heavy metals in RDS were measured and the result of this experimental study suggested that the metal concentrations in seawater soluble fraction increase along with salinity of seawater and duration during the sinking process in water column.

SPATIAL DISTRIBUTION OF ENVIRONMENTAL PARAMETERS IN FUTIAN MANGROVE FOREST BASED ON THE HEALTH ASSESSMENT SYSTEM

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The Futian mangrove forest in Shenzhen bay is the only one which grows in metropolis interiorly with the characteristics of small area, fragile ecosystems and serious disturbance by human activities. So it is urgent to establish dynamic monitoring and ecological health assessment system to enhance its protection and management. A health assessment system of Futian mangrove forest is established on the basis of GIS, including the best selection of sampling points, the diversity index analysis, the potential risk assessment the spatial distribution of environmental factors, namely pH, conductivity, chemical oxygen demand (COD), biological oxygen demand (BOD₅), dissolved oxygen (DO), oxidation reduction potential (ORP), total phosphorus (TP), total nitrogen (TN), salinity, and turbidity of the water, and other analysis functions. The changes of the environmental factors and the diversity of the zooplankton with space and time are studied. The Results based on the system showed that the environmental factors varied significantly on different seasons and regions, the COD, BOD5, and the diversity of the zooplankton at the buffer zone are higher than Shenzhen river, the core zone of the mangrove forest nature reserve, indicating the buffer zone is seriously polluted and it's health risk is relatively high. These results are consistent with the current situation, that the human activities generate continuous contaminants which flows into the buffer zone leading to water pollution. The system can correctly reflect the actual information and is applicable in the Futian mangrove forest.

OCCURRENCE AND ASSESSMENT OF PERFLUOROALKYL ACIDS IN COMMONLY CONSUMED SEAFOOD FROM THE COASTAL AREA OF BANGLADESH

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This study reports the first evidence of the occurrence of perfluoroalkyl acids (PFAAs) in the seafood collected from the coastal area of Bangladesh (a tropical ecosystem). A total of 48 seafood samples (5 finfish and 2 shellfish species) were collected from four major coastal areas of Bangladesh (Cox's Bazar, Chittagong, Bhola, and Sundarbans) in winter and summer of 2015. Finfish and shellfish samples were digested with a basic solution of 10 mM sodium hydroxide in methanol before sonication and solid phase extraction through weak anion exchange followed by a dispersive carbon sorbent clean-up. Fifteen target PFAAs, including: C₄₋₁₄-perfluoroalkyl carboxylates (PFCAs), and C4, C6, C8, and C10-perfluoroalkyl sulfonates (PFSAs) were quantified by high performance liquid chromatographytandem mass spectrometry (HPLC-MS/MS). The Σ PFAAs in finfish and shellfish samples were in the range of 0.32– 14.58 ng/g wet weight and 1.31-8.34 ng/g ww, respectively. Perfluorooctanesulfonate (PFOS) was the dominant PFAA in both finfish and shellfish, whereas perfluorooctanoic acid (PFOA) was the predominant PFAA in shellfish, which were comparable with the most other values reported worldwide, particularly from China, Spain, Sweden, and USA. Concentrations of PFOS ranged from 0.1 to 3.86 and 0.1 to 1.99 ng/g ww in finfish and shellfish, respectively, with the highest concentration in Hilsa shad from Chittagong. Concentrations of PFOA in shellfish ranged from 0.07 to 2.39 ng/g ww, with the maximum concentration found in crab also from Chittagong. The monitoring results for the seafood samples showed no obvious seasonal variations. Spatial distribution revealed that the seafood of the southeast part (Cox's Bazar and Chittagong) of the Bangladeshi coastal area was more contaminated by PFAAs than the south (Bhola) and southwest part (Sundarbans). Furthermore, diet is an important source of PFAA exposure and seafood is the major dietary component for the coastal populations of Bangladesh. Therefore, it is an urgent need to assess the potential health risk that might be contributed from the dietary exposure of PFAAs through seafood consumption. The estimated dietary intakes (EDI, ng/kg body weight/day) of PFOA, PFOS and total PFAAs for the adult and children were calculated by multiplying the mean concentrations (ng/g ww) of PFOA, PFOS and total PFAAs in seafood with the daily consumption data (g/day). The body weight and seafood consumption data for the adult and children were derived from the dietary questionnaire survey carried out in the four studied coastal areas of Bangladesh. The highest EDI of PFOA and PFOS were found to be 0.73 ng/kg-bw/day and 1.89 ng/kg-bw/day for adult and 1.02 ng/kg-bw/day and 2.08 ng/kg-bw/day for children, respectively. However, the highest EDI of total PFAAs was found at 7.78 ng/kgbw/day for adult and 9.01 ng/kg-bw/day for children, both of which were from Chittagong. The EDI from seafood is much lower than the tolerable daily intake (TDI) recommended by the European Food Safety Authority in 2008 (PFOA: 1500 ng/kg-bw/day; PFOS: 150 ng/kg-bw/day), indicating low health risk of PFAAs exposure via consumption of seafood among the coastal populations in Bangladesh.

CONSERVING INTERTIDAL HABITATS: WHAT IS THE POTENTIAL OF ECOLOGICAL ENGINEERING TO MITIGATE IMPACTS OF COASTAL STRUCTURES?

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Growing human coastal populations and increasing urbanisation are concurrent with climate change which brings stormier seas and rising tides. These trends create a strong and sustained demand for land reclamation and coastal protection, requiring coastal engineering such as seawalls. In response, research examining the ecological impacts of coastal engineering and potential mitigations has increased over the past 15 years. Through review, we synthesise current understanding of three key areas of this burgeoning research field: i) ecological impacts of coastal engineered structures on intertidal ecosystems ii) current status of ecological engineering to mitigate such impacts iii) effectiveness of mitigation as a tool to contribute to conservation of intertidal habitats. Engineered structures alter important physical, chemical and biological processes of intertidal habitats, and strongly impact community structure, inter-habitat linkages and ecosystem services while also driving habitat loss. Such impacts occur diffusely across localised sites but are significant at regional and global levels. Ecological engineering includes small-scale artificial habitat provision on hard structures; the inclusion of natural materials, species or processes in hybrid 'soft' structures; and increasingly, large-scale habitat restoration or managed realignment that delivers natural coastal protection services. Soft solutions and natural habitats maximise multiple services, providing greater economic benefits for society and resilience to climatic change. Currently however, under-inclusion and economic undervaluation of intertidal ecosystem services may undermine best practice in coastline management. Importantly, reviewed evidence shows mitigation and even restoration do not support intertidal communities or processes equivalent to pre-disturbance conditions. Crucially, a lack of ecological baseline data (including ecosystem functions and services) prohibits quantification of relative and absolute impacts to intertidal habitats from coastal structures and the effectiveness of mitigations, restricting development of conservation policy. To improve mitigation design and effectiveness, a greater focus on *in-situ* research is needed, requiring timely collaboration between government, construction partners and scientists.

CONCENTRATION OF ORGANOTIN AND ALTERNATIVE BIOCIDES IN SEDIMENTS OF SUNGAI PULAI SEAGRASS AREA, MALAYSIA

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Antifouling biocides have been found to cause various effects on targeted and non-targeted organisms. Sungai Pulai seagrass area is located very close to active ports, thus exposed to contamination by antifouling biocides. Present study aimed to determine concentration of organotin and alternative biocides in sediments of Sungai Pulai seagrass area. Samples of sediments were collected from a few predetermined points within the seagrass area. Samples of organotin and other biocides were conducted based on the established extraction procedures. Results showed concentrations of selected antifouling biocides were as follows; monobutyltin-MBT (6.4 to 12.3 μ g/kg), dibutyltin-DBT (n.d. to 6.1 μ g/kg), tributyltin-TBT (8.0 to 10.6 μ g/kg), monophenyltin-MPT (n.d. to 16.1 μ g/kg), Dichlofluanide (48.6 to 800.4 μ g/kg), Chlorothalonil (0.004 to 6.3 μ g/kg), Irgarol (n.d. to 1.5 μ g/kg), M1 (44.0 to 876.7 μ g/kg) and Sea Nine (9.1 to 169.7 μ g/kg). Comparison with previous data revealed that butyltin concentrations were significantly reduced, phenyltin concentration of species still within previous ranges, and alternative biocides concentration were drastically increased. Continuous monitoring of these biocides in marine environment is crucial to prevent serious effects on stability and richness of marine ecosystem.

DISTRIBUTION OF HEAVY METALS CONTENT IN SURFICIAL SEDIMENT IN COASTAL VICINITY OFF MALACCA STRAITS

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The paper presents distribution of heavy metal content in surface layer sediments off coast of Peninsular Malaysia of Malacca Straits region. Teflon bomb digestion method applied and samples were detected by Inductively coupled plasma spectrometry (ICPMS). The distribution is then correlated with its mean size (\emptyset) and silt and clay percentages. There is a moderate linear relationship between Li (r= 0.718) and Zn (r= 0.608) and mean size. There were also moderate relationship when compared to silt & clay percentages for Li (r= 0.819) Zn (r= 0.653) Cu (r= 0.653). The area is dominated by fine sediments north of the area, around Pulau Langkawi other than at Pinang vicinities. Normalization with Li and enrichment factor (EF) are used in the assessment of heavy metal pollution in area. Enrichment ranked Al> Fe> Mn> Ni> Co> Hg> Zn> Cr> Cd> Pb> Cu> As. The moderate enrichment input indicated anthropogenic input while rest of metals in sediments were from natural sources. Multiple factors could influence the distribution include continuous anthropogenic input, water current of Selat Melaka, and sediment size.

ASSESSMENT OF HEAVY METALS IN THE MUSCLES AND FINS OF FOUR LANDED SHARK FROM KUALA TERENGGANU WATERS

MCOng and SLGan

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A study had been carried out to determine the heavy metals (Cu, Zn, Cd, Hg and Pb) concentrations in the muscle and fins of four different shark species, purchased from LKIM Complex, Pulau Kambing, Kuala Terengganu, Terengganu, Malaysia. Species of shark used in this study includes spot-tail sharks (Carcharhinus sorrah), milk sharks (Rhizoprionodon acutus), whitespotted bamboo sharks (Chiloscyllium plagiosum) and also whitespotted guitarfish (Rhynchobatus australiae). After the detection by Inductively Coupled Plasma Mass Spectrometry (ICP-MS), Zn level was found to have highest concentration whereas Cd had the lowest concentration in both studied organs. Levels of heavy metals in this study were also compared with the guidelines set by Malaysian Food Regulation and also Turk Gida Kodeksi / Turkey Food Codex. Provisional Tolerable Weekly Intake (PTWI) for human consumption of four landed shark from Kuala Terengganu Waters was also determined. It can be concluded that the consumption of the muscle of three landed shark species from Kuala Terengganu Waters of an adult are not safe due to a high amount of mercury found.

METALLOTHIONEIN-LIKE PROTEIN LEVELS AND HISTOLOGICAL STUDIES IN JAVANESE MEDAKA (*ORYZIAS JAVANICUS*) EXPOSED TO DIFFERENT CONCENTRATIONS OF ZINC PYRITHIONE (ZnPT)

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The uses of organotin biocides have been completely banned utilized as active biocides in antifouling paints since the early 1960s. The effectiveness of Zinc pyrithiones (ZnPT) against bacteria, fungi and algae make them as one of the organic biocides replacements for organotin compound in antifouling paints. ZnPT also widely used as active ingredients in anti-dandruff shampoos or as additive in cosmetics and dermatitis treatment. ZnPT may introduce into the aquatic environment through the municipal household wastewater and harbor activities. Environmental fate studies show that ZnPT rapidly degrade in the water column to less toxic compound due to photolysis process. However, recent studies have suggested that ZnPT can persist in the marine environment, especially where the sources of light is limited. This study investigates the ecotoxicological effect of zinc pyrithione in a new model fish species known as Javanese medaka (Oryzias javanicus) under sublethal level and the effects of ZnPT exposure on the gonads via histological studies. Besides, induction of metallothione (MT) and levels of zinc pyrithione are studied for 21days exposure of adult fish to different concentration of ZnPT. Correlation between ZnPT and MT in *O. javanicus* are statistically significant and correlation is positive; the ZnPT and MT concentrations in O. javanicus indicate the increase in ZnPT levels is followed with the increase MT levels also.

DE-TOXICITY AND RECYCLE OF HAZARDOUS INCINERATOR FLY ASH WITH HARBOR SEDIMENT AND OYSTER SHELL

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Harbor sediment dredged from Taichung harbour in central Taiwan proves to be effective in detoxicity of hazardous incinerator fly ash at elevated temperatures through metallurgical phase conversion and immobilization of toxic component. Disposal cost of the hazardous fly ash is about four times the de-toxicified fly ash. Further, thermal treatment of the mixture generates lightweight aggregates meeting European Union regulation UNIEN 13055-1 for construction engineering purpose. The sintering reaction is conducted within the range of 1000°C and 1200°C for 10-20 minutes. Mixing ratios are 60-90% harbour sediment with the fly ash and waste oyster shell powders as a balance. Richness in calcium, greater than 20% in the incinerator fly ash and 40% in oyster shell, is beneficial to sintering because of calcium's flux characteristics. Leaching experiment shows that leached toxic metals, such as lead, cadmium, and others are below legal threshold formulated by USEPA. The 24-hour water sorption rate of the lightweight aggregates can meet practical requirement, 20%, for construction industry. Compressive strength of the lightweight aggregates generally decreases with increasing temperature.ICP-MS, XRF, XRD, SEM, TGA, particle size analyser, and ion chromatograph are used to characterize physical and chemical properties of raw materials and the sintered product. Lightweight aggregates can be produced in present study at 1050°C–1150°C that is generally 150°C lower than commercially available lightweight aggregates, saving considerable amount of energy while recycling the hazardous ash same time. The lightweight aggregate product can be applied to horticulture for holding waster, to environmental engineering for growing bacteria in waste water treatment facility, and to non-structural construction purpose, such as room partition, road pavement, parking lot aspect, and others.

GENOME-WIDE TRANSCRIPTIONAL INVESTIGATION OF MOLECULAR REPONSES TO CADMIUM EXPOSURE OF HEPATOPANCREAS IN THE FRESHWATER CRAB SINOPOTAMON HENANENSE

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Cadmium (Cd), a widespread pollutant in aquatic system, can cause various toxic effects in animals. Freshwater crabs, one of the widely distributed decapod, are useful environmental bioindicators. It has been demonstrated that Cd stress resulted in alterations of the tissue histological structures and impairments of various physiological functions in the crabs. However, little is known about the molecular events produced by heavy metal toxicity due to the lack of genomic information.

In the present study, we performed the transcriptomic sequencing of the pooled samples from hepatopancreas of the crabs and digital gene expression (DGE) profile analysis with and without Cd exposure. De novo sequence assembly generated a total of 180,318 contigs with a mean length of 279 bp which further assembled into 68,648 unigenes with an average size of 622 bp. From here, 10,675 unigenes were classified into 56 functional terms by Gene Ontology annotation, and 17,169 unigenes were directly assigned to 258 KEGG pathways. We identified 5,436 metabolismassociated unigenes and 2,728 stimulus response-associated unigenes. Among the latter group, 380 unigeness were associated with the regulation of immunoresposne. Four gene expression profiles were constructed and analyzed from the samples treated with 0 (control), 7.25, 14.5 and 29.0 mg/L Cd for 48 hours respectively. Compared with the controls, 4,055, 4,166 and 4,072 differentially expressed genes (DEGs) were obtained from the treatment groups (7.25, 14.5 and 29 mg/L Cd) respectively, among which 3,265 DEGs were commonly detected, 501 DEGs being upregulated and 2,764 DEGs downregulated. Major of these DEGs are involved in metabolism and response to stimulus, and the expression pattern and the number of DEGs are correlated to the different concentration of Cd. Therefore, the large-scale sequence information yielded in our study provides the genomic foundation for biological and ecotoxicological studies on the crab and its related species in decapod. These DEGs and pathways related to Cd toxicity can be used for gene discovery and biomarker identification for monitoring aquatic pollution by heavy metals such as Cd.